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Volume 36 *Number 2*

Phytopathology

AN INTERNATIONAL JOURNAL

February 1946

53

Official Organ of

The American Phytopathological Society

$6.25 per year, Canada $5.00 per year, United States and dependencies $6.50 per year, elsewhere

Office of Publication

Corner North Queen Street and McGovern Avenue, Lancaster, Pennsylvania

Entered at the postoffice in Lancaster, Pennsylvania, as second-class matter.

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An International Journal
Official Organ of The American Phytopathological Society

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EDWARD FRANKLIN GAINES

1886–1944

HARRY B. HUMPHREY AND FREDERICK J. STEVENSON

Edward Franklin Gaines was born January 12, 1886, near Avalon, Missouri. When a lad of fourteen the family moved from Missouri to a farm near Chewelah, in the Colville Valley of northeastern Washington. It was here and in Missouri that he received his public school education. Thereafter he attended the State Normal School at Cheney, Washington, where he was graduated in 1907. We next find him as the principal of a grade school in Ritzville. In 1909 he entered the State College of Washington, at Pullman, where, in 1911, he received the degree of Bachelor of Science in Agriculture.

On graduation the subject of our narrative was appointed to a position on the Experiment Station staff, as assistant cerealist. The nature of this appointment enabled him to continue his studies to the end that in June, 1913, he was awarded the M.S. degree. We next find him for a semester in 1915 at Harvard University as a graduate student of genetics and plant breeding under the leadership of the late Dr. E. M. East. Returning to Pullman, he resumed his duties as assistant cerealist, which position he filled until 1917, when he was promoted to the rank of cerealist. In 1920 he was again granted leave for resumption of his postgraduate studies at Harvard, where, in 1921, he received the degree of Doctor of Science.

By way of partial acknowledgment of the outstanding quality and character of Dr. Gaines as student and man, it suffices here to mention the fact that, while at Pullman, he was elected to membership in Alpha Gamma Rho, Alpha Zeta, Phi Kappa Phi, Sigma Xi, Phi Sigma, and Phi Beta Kappa. Of these honors and badges of student esteem and distinction he was justly proud, yet he was ever the same wholesomely modest and genuine student, and inspiring teacher.

His ability as a teacher was early recognized, and he served his alma mater eminently, first as instructor in agronomy (1911–1917), then as assistant professor of farm crops (1917–1921), and associate professor of farm crops (1921–1930), and finally as professor of genetics in agronomy, from 1930 until his death. Because of illness, he was unable to be at his desk during the last nineteen months of his life.

The last six months of 1930 Dr. Gaines spent in Europe, where he visited experiment stations and educational institutions in Great Britain, Russia, Germany, Sweden, and Denmark. Incidentally, while in Europe, he attended the Fifth International Botanical Congress, at Cambridge, and the Second International Congress of Soil Science, held that summer in Leningrad and Moscow. In early October he took up residence in Halle, Germany, seat of the Institute for Plant Breeding and Plant Research.

EDWARD FRANKLIN GAINES
1886–1944

Here he visited and made observations on the several field experiments and had access to the records. He also made several excursions into the surrounding country, where he visited private plant-breeding centers, outlying stations of the Institute, centers of beet-sugar production, and several large, privately owned farms.

Most important among Dr. Gaines' contributions as a geneticist and plant breeder are his papers on the genetics of bunt resistance in wheat and his studies on the inheritance of smut resistance in wheat and oats. The appended list of his published research will afford the reader a ready grasp of the range and importance of his scientific contributions to our knowledge of agronomy, and of genetics as applied to breeding for disease resistance.

During his long term of service as a teacher students came to Dr. Gaines from Canada, Belgium, Germany, the Philippines, China, and Japan to study under his supervision. He was visited by geneticists from every continent, men who desired particularly to learn of his methods and results.

To his intimate friends "Ed" Gaines was more than a great scientist. He was a great man. He believed in mankind and in his social betterment, and was always to be found in the front ranks of character-building organizations. He worked with young people in the Boy Scouts and in the Church, with students in various college organizations, especially in the Alpha Gamma Rho fraternity, and with farmers, in whose welfare he always showed, through the Grange, an intense interest.

He radiated trustworthiness and steadfastness of purpose. He had the ability to undertake worthwhile projects and carry them through, often in spite of formidable opposition.

Those students privileged to work under his direction were treated not as inferiors but as close friends whom he inspired with his own zeal for science and right living. He was not an outstanding lecturer, but he was an eminent teacher and leader. Not many words but more doing seemed to be his motto.

His scientific achievements will long be remembered, but his influence for good over many lives, while not so easily evaluated, probably will result in greater gains to society than the results of his scientific work. We can truly say that the Pacific Northwest and the whole world are enriched physically and morally because Ed Gaines passed this way.

A LIST OF THE PUBLISHED PAPERS OF WHICH DR. EDWARD F. GAINES WAS EITHER AUTHOR OR CO-AUTHOR

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The inheritance of reaction of Turkey-Florence-1 × Oro-1 to Race 11 of *Tilletia tritici*. (With O. A. VOGEL and C. E. CLAASSEN.) Jour. Amer. Soc. Agron. **36**: 473–479. 1944.

CHEMICAL CONTROL OF SEED-BORNE FUNGI DURING GERMINATION TESTING OF PEAS AND SWEET CORN[1]

WILLARD CROSIER[2]

(Accepted for publication July 10, 1945)

In routine germination testing in the laboratory seeds usually are placed close together on moistened paper towels. Since the atmosphere in the germinators is very humid, bacteria and fungi grow rapidly and spread quickly from infected to noninfected seeds and seedlings. Because the seeds are crowded, decays caused by seed-borne pathogens may be very severe in the germinator. *Rhizoctonia solani* Kühn originating in a few infected beans or peas (4) often grows throughout, and rots most of the seeds in, a rolled towel in 5 to 7 days. Molds frequently develop in lots of beans, corn, and peas (4, 5), and especially in seeds of low vitality, to such an extent that the seedlings become hidden and can be observed only with difficulty.

The fungicides commonly applied to seeds prior to planting (2, 5, 6, 7) have been used to control both pathogens and saprophytes troublesome in laboratory germination testing. Hay (5) reported that certain organic mercurials controlled the molds on bean seeds very satisfactorily and that the laboratory germination was uniformly higher for treated than for untreated seeds. Porter (9) found that the volatile, water-soluble mercurials were very effective as laboratory seed disinfectants and disinfestants. Crosier and Patrick (4) concluded that mercuric chloride, and dusts containing ethyl mercury phosphate, as well as several insoluble organic mercurials prevented mold growth if the germination periods did not exceed 10 days.

Certain copper compounds including copper oxalate, copper oxychloride, and cuprous oxide were not so effective as the mercurials in short-period tests. Buchholtz (2) stated that the chemicals (copper carbonate and ethyl mercury phosphate) recommended for control of sorghum smut also killed molds that developed on seeds during storage. Patrick and Crosier (8) found that any one of several mercurials, when used either as dip or dust, controlled *Alternaria spp.* and *Rhizopus nigricans* Ehr. in germinating cabbage and radish seed. Cuprous oxide and zinc oxide, however, caused only slight reductions in the percentages of fungus-seed associations. Hoppe (6) found that Arasan reduced the *Diplodia zeae* (Schw.) Lév. on infected corn seed but that Spergon was ineffective in control of this fungus. Muskett and Colhoun (7) reported that Arasan (or Nomersan) eliminated *Colletotrichum lini* (Westerd.) Toch. and partially controlled *Polyspora lini* Laff. in flax seed. They concluded that the organic mercurials were effective only when used as short wet treatments.

[1] Journal Paper No. 610, New York State Agricultural Experiment Station, Geneva, N. Y.

[2] Assistant Professor, Division of Seed Investigations, Cornell University, Geneva, N. Y.

METHODS AND MATERIALS

The present investigation was undertaken to determine the relative fungicidal values of Arasan, Ceresan, du Pont 1452 C, Semesan Jr., Spergon, and United States Rubber Compound No. 604. All chemicals were applied by agitating weighed amounts of dust with measured quantities of seeds in glass jars secured to a rotating wheel treater. The treatment periods were uniformly 5 minutes. A quantity of Stowell's Evergreen Sweet Corn grown in 1942 contained about 8 per cent of seeds infected with *Diplodia zeae*. *Rhizopus nigricans* and species of *Aspergillus, Fusarium,* and *Penicillium* occurred as contaminants on 50 to 60 per cent of the seeds. This lot of sweet corn seed was treated with certain fungicides at the following rates: Arasan (50 per cent tetramethyl thiuramdisulfide), 0.15 per cent by weight (1¼ oz. per bu. of seed); Du P. 1452 C (7.7 per cent ethyl mercury p-toluene sulfonanalide), 0.06 per cent by weight; Semesan Jr. (1 per cent ethyl mercury phosphate), 0.22 per cent by weight; U.S.R. No. 604 (essentially 2,3-dichloro-1,4-naphthoquinone), 0.11 per cent by weight. The seeds were germinated in rolled paper towels, placed in humid germinators with either constant temperatures of 25° C. or alternating temperatures of 20° and 30° C. The seedlings were removed and the fungicidal treatments were evaluated 6 days after initiation of the tests.

A sample from the 1942 crop of Thomas Laxton peas was separated into six portions and treated with chemical dusts applied in percentages by weight of the seeds as follows: Arasan, 0.15 per cent (1.44 oz. per bu.); Ceresan (2 per cent ethyl mercury chloride), 0.20 per cent; Semesan Jr., 0.20 per cent; Spergon, 0.20 per cent; and U.S.R. No. 604, 0.10 per cent. The seeds were germinated at 20° C. Records were taken the sixth day of germination.

The treated lots of peas and sweet corn were separated into 100-seed samples and distributed in triplicate to 11 official seed analysts in New York and other states for germination and disease studies. The peas were also planted at Geneva, N. Y., in greenhouse soil infested with *Pythium ultimum* Trow., *Rhizoctonia solani,* and *Fusarium spp.* The treated peas were planted in 5 replicates of 100 seeds, the untreated peas in 10 replicates of 100 seeds. After 25 days the emerged seedlings were counted, examined, and cut at the soil line, and the green tops were weighed.

RESULTS

Since the sweet corn seedstock was heavily infested with *Rhizopus nigricans* several cooperating analysts stated that the untreated germinated seeds were entirely obscured by growths of this fungus. Neither the statements nor the data of any analyst indicated that *R. nigricans* depressed the actual germination. This is in accord with previous findings at the New York laboratory. In experiments over a period of six years seedstocks infested with *R. nigricans* alone have not produced more seedlings from treated than from untreated seeds. Control of *R. nigricans,* however, may be accom-

panied by an increase in the green weights of the germinating seeds and seedlings. The results of the present investigation (Table 1) with a stock of sweet corn in which *Diplodia zeae* was also a factor suggest that increased weight of seeds and seedlings is obtained from even a partial control of molds.

Diplodia zeae grew rapidly on the untreated infected seeds and destroyed or injured from 3 to 10 per cent of the seedlings. Material was examined

TABLE 1.—*Influence of certain seed protectants upon seed germination, incidence of fungus contamination or infection, and the green weight of Stowell's Evergreen sweet corn seedlings*

Seed protectant	Germination		Seeds or seedlings with growths of						Green weight of seedlings	
	Normal	Total	*Rhizopus nigricans*		Pen., Asp., and Horm.[a]		*Fusaria*[b]		*Diplodia zeae*			
	Pct.	*No.* *Pct.*	*No.*	*Pct.*	*No.*	*Pct.*	*No.*	*Pct.*	*No.*	*Pct.*	*No.*	*Gm.*
Arasan												
Times first[c]		2	2		3½		3¾		7½		1	
Times excelled[d]		29	22½		29½		28		40½		19	
Average	68	78		28		2		8		2		66
Du Pont 1452 C												
Times first		4½	6		2		2½		3		8	
Times excelled		29½	37		25½		30		29		39½	
Average	71	78		24		5		6		4		70
Semesan Jr.												
Times first		3	2		2½		¼		½		1	
Times excelled		27½	20½		24		19		20½		25	
Average	70	78		32		6		8		5		66
U.S.R. No. 604												
Times first		1½	1		3		3½		0		0	
Times excelled		23	27		31		29		20		15½	
Average	69	77		28		2		5		6		65
No treatment												
Times first		0	0		0		1		0		1	
Times excelled		1	3		0		6		0		11	
Average	60	74		56		14		17		15		60

[a] Species of *Penicillium*, *Aspergillus*, and *Hormodendrum*.

[b] *Fusarium moniliforme* was the most frequently observed species.

[c] Number of times this material excelled all other materials and the check, 11 possibilities. Ties of 1 material with 1 to 4 others are recorded as ½ to ⅕, respectively.

[d] Number of times this material excelled one of the other materials or check, 44 possibilities for each material.

before the hyphae spread extensively throughout the rolled towels, hence the injury from *D. zeae* was limited to the infected seeds and those immediately adjacent. The increased percentages of germination (Table 1) in the treated 100-seed lots are undoubtedly attributable to the chemical control of *D. zeae*.

Only U.S.R. No. 604 effected more than a trace of chemical injury. The average percentages of germination from the seeds treated otherwise (Table 1) indicate a general absence of phytotoxicity. The organic mercurial, Du P. 1452 C, was outstanding in the control of molds and *Diplodia zeae*, although U.S.R. No. 604 and Arasan were nearly as fungicidal.

The seeds treated with Du P. 1452 C produced a greater weight of sprouts than seed treated with any other material in trials by eight cooperators. The weight of the sprouts detached from the endosperms and seedcoats averaged 54.3 grams for the 100 seeds treated with Du P. 1452 C or a gain of 23 per cent over the 44.3 grams for the untreated seed. The plumules and radicles frequently were larger in the former tests. A heavier production of lateral

TABLE 2.—*Influence of certain seed protectants upon seed germination, fungus- and bacterial-contamination and green weight of Thomas Laxton pea seedlings*

Seed protectant	Germination			Seeds or seedlings with growth of						Green weight of seedlings	
	Normal	Total		*Rhizopus nigricans*		Pen., Asp., Fus., etc.[a]		Bacteria[b]			
	Pct.	*No.*	*Pct.*	*No.*	*Pct.*	*No.*	*Pct.*	*No.*	*Pct.*	*No.*	*Gm.*
Arasan											
Times first[c]		1½		⅚		2		0		5⅓	
Times excelled[d]		32		31½		33		22½		44½	
Average	87		93		2		1		6		116
Ceresan											
Times first		3		⅚		2½		2½		1½	
Times excelled		31		26½		34½		32½		34	
Average	89		95		4		1		5		114
Semesan Jr.											
Times first		1		2⅓		1		3		⅓	
Times excelled		19		31½		21		26½		17½	
Average	86		93		3		3		5		107
Spergon											
Times first		3½		3⅚		1½		½		1½	
Times excelled		29½		41		36		27½		24½	
Average	88		94		2		1		5		111
U.S.R. No. 604											
Times first		2		1⅓		3		3½		1½	
Times excelled		34		33½		40½		38		22½	
Average	88		95		2		1		3		111
No treatment											
Times first		½		0		0		1½		⅚	
Times excelled		18½		0		0		18		22	
Average	85		95		9		8		6		108

[a] Species of *Penicillium, Aspergillus, Fusarium,* and other fungi.

[b] *Erwinia carotovora* caused most of the soft-rotting.

[c] Number of times this material excelled all other materials and the check, 11 possibilities. Ties of 1 material with 1 to 5 others are recorded as ½ to ⅙, respectively.

[d] Number of times this material excelled one of the other materials or check, 55 possibilities for each material.

roots also was observed. The average weight per detached seedling was also greatest for the lots treated with Du P. 1452 C. Since the stock of peas used in this study contained very few dead, weak, or contaminated seeds, the control of molds and bacteria was complete in several tests and nearly so in the others. Each cooperator reported that the incidence of *Rhizopus nigricans* and of *Penicillium spp.* was less in every treated lot than in any untreated lot of seeds. Spergon and U.S.R. No. 604, excelled in the control of mold fungi (Table 2) while the latter also ranked first as a protectant against

bacterial contaminations. The chemical control of the bacteria and fungi greatly facilitated the examination of the germinant seeds.

It is obvious that growth of bacteria and fungi in the untreated seed was too limited to depress the germination greatly. For this reason the treated seeds produced only slightly more normal and total sprouts than did the untreated. Unfortunately the stock of peas was not infected with *Ascochyta pisi* Lib., *Rhizoctonia solani*, or *Sclerotinia sclerotiorum*, so the value of the chemicals in eliminating these pathogens was not determined.

In 55 comparisons with other protectants (Table 2) Arasan excelled, or tied, 46 times on the basis of total green weight from 100 seeds and seedlings. Each cooperator who measured the length of radicles and plumules reported that Arasan excelled all other treatments. By the same criterion Ceresan appeared to be only slightly inferior to Arasan both in the present study and in former experiments with similar seedstocks. Ceresan had previously

TABLE 3.—*Emergence and green weight of tops of plants from treated and from untreated pea seed planted in contaminated greenhouse soil*

Seed protectant	Plants emerged in 25 days			Green weight of tops	
	Normal	Weak or infected	Total	Total	Gain[a]
	Per cent	*Per cent*	*Per cent*	*Grams*	*Per cent*
Arasan	85	4	89 ± 5	116	332
Ceresan	39	11	50 ± 8	66	144
Semesan Jr.	37	5	42 ± 3	50	85
Spergon	86	3	89 ± 9	120	348
U.S.R. No. 604	76	6	81 ± 3	103	283
None	26	8	34 ± 7	27	.....

[a] The weight of plants from untreated seeds equals 100 per cent.

(4) excelled the copper compounds in this respect with germination tests of peas terminating within seven days.

In greenhouse beds both Arasan and Spergon were slightly superior to U.S.R. No. 604 (Table 3) in the protection of pea seeds and seedlings against damping-off. The water-soluble ethyl mercurials, Ceresan and Semesan Jr., did not compare favorably with the former materials as seed protectants. It should be noted that Arasan was applied at only three-fourths and U.S.R. No. 604 at one-half the weight dosage of the other three materials. The plants in each of the replicates from treated seed were larger and more uniform than in those from untreated seeds. The average weight per plant (Table 3) indicates that each of the materials protected the emerged seedlings from root-rot and other fungus injury. The plants from the Arasan- and Spergon-treated seeds were taller and were maturing more rapidly than those grown from the peas left untreated or treated with other materials.

DISCUSSION

The organic mercurials, Ceresan and Semesan Jr., applied to seed corn prior to planting have effected practical control of molds in laboratory

germination techniques. Low-germinating stocks of seed, such as the sweet corn seed used in this study, may be so infested with sub-surface growths of *Rhizopus nigricans,* however, that seed treatment does not eliminate all of this fungus. The dosages of the seed protectants may be considered minima for application to normal planting stocks of sweet corn. Slightly greater amounts of Semesan Jr. will adhere to the seed coats and are usually recommended, while the dosages of the other materials could be increased from 2 to 5 times. However, with the exception of Arasan and Spergon, and possibly of Du P. 1452 C, such increases in dosage would be accompanied by definite phytotoxicity. It would appear then that any of the materials used in this study could be applied in sufficient amounts to control *R. nigricans* and other fungi but that Arasan alone would effect a high degree of control of both pathogenic and saprophytic fungi without injury to the seedlings. Arasan has frequently been applied without dilution to corn seed and the seedlings developed normally. Usually the adherence rates varied from 0.30 to 0.45 per cent by weight with an average of 0.35 per cent (3.1 ounces per bushel of seed).

Under certain conditions the maximum adherent dosage of Arasan may be necessary to inhibit *Diplodia zeae.* Hoppe (6) employing a technique which permitted maximum adherence of dusts to corn seed found Arasan about as efficient as New Improved Semesan Jr. or Barbak D in the inhibition of *D. zeae.* Since the dosage of Arasan used by Hoppe was probably 50 to 100 per cent heavier than those of the other dusts it may be inferred that equal dosages would prove Arasan to be relatively less efficient. Repeated experiments at the New York State laboratory, however, indicate that the application of a mixture of equal parts of Arasan and talc (only 25 per cent tetramethyl thiuramdisulfide) to infected corn seed inhibits the growth of *D. zeae.*

The material Du P. 1452 C is, in certain respects, preferable to Arasan for laboratory practice, since it can be used as an instant dip. The consistently high green weight of seedlings (Table 1) from Du P. 1452 C-treated seed has been observed repeatedly in other experiments. It is not uncommon for the seedlings from each test of treated seed in a series of 20 samples to outweigh the germinated seed in the untreated control by 5 to 10 per cent. When applied to commercial seed usually less heavily contaminated than the stock of Stowell's Evergreen sweet corn used in this trial, a 0.20 per cent suspension of Du P. 1452 C in water or a 20 per cent Du P. 1452 C–80 per cent flour mixture practically eliminated the *Rhizopus* and *Penicillium* and inhibited the development of *Diplodia zeae* and *Gibberella zeae* (Schw.) Petch.

In the control of molds U.S.R. No. 604 is about equal to Arasan at the dosages used in this study. At equal dosages U.S.R. No. 604 might prove the better. However, while Arasan causes no seedling injury at heavy dosages, U.S.R. No. 604 appears to be phytotoxic if the dosage exceeds 0.11 per cent by weight. In the control of *Diplodia zeae* this material is definitely inferior

to Arasan and to the organic mercury dusts. In this regard U.S.R. No. 604 appears similar to the related quinone compound, Spergon, which did not inhibit the growth of *D. zeae* on infected seed (6).

New Improved Ceresan, one of the standard disinfestants for pea seeds, can be applied in either dip or dust form and is effective at dosages only 20 to 40 per cent as heavy as for most other materials. Semesan Jr. at five times the dosage rate of New Improved Ceresan has often been substituted for it since Semesan Jr. contains the same fungicidal ingredient and adheres at the correct rate to control seed contaminants without producing seedling injury.

Ceresan and New Improved Ceresan have been of some value in controlling *Ascochyta pisi* Lib. in seedstocks of peas and winter vetch (3, 4). Bayles *et al.* (1) also found that the organic mercurials reduced the amount of seed-borne *Ascochyta*, while cuprous oxide was ineffective.

Since the advent of Spergon as a seed protectant for peas, a mixture of 60 per cent Spergon and 40 per cent flour by weight has been used in the laboratory. Such a dust adheres at a rate equivalent to 2 to 4 ounces of Spergon per bushel of seed. The results of this study indicate that Spergon applied at about this dosage will be equal or even superior to Semesan Jr. in the control of molds. Spergon seemed to cause no discomfort to the worker when treating seeds, while the other four dusts applied to pea seeds in this investigation caused slight irritation. Seedstocks of pea treated with Spergon before their receipt at the seed testing laboratory usually produced strong clean seedlings on paper towels. Heavily dusted seeds have germinated normally although the coverages indicated that Spergon had been applied at rates of 5 to 8 ounces per bushel.

Arasan has eliminated molds from germinant pea seeds treated at the canning factory, and Arasan has been practically equal to Spergon in this respect. Similar studies with cabbage seed (8) have indicated that both Arasan and Spergon are about equal to Semesan in control of *Alternaria spp.* and *Rhizopus nigricans.*

SUMMARY

Every chemical preparation used in this study, and especially Du P. 1452 C, inhibited, or partially controlled, the seed-borne fungi of sweet corn in germination testing. Increases in germination attributable to seed treatment paralleled the control of *Diplodia zeae* but probably not the control of *Rhizopus nigricans.*

Only U.S.R. No. 604 caused any chemical injury detectable by size or development of the sweet corn seedlings. Every other material resulted in increased green weight of the combined seeds and seedlings. Du P. 1452 C excelled or equalled the other chemicals in 41 of 44 possibilities on the basis of green weight. Arasan was the most, and U.S.R. No. 604 the least, efficient material in preventing *Diplodia zeae* from developing on seeds and from destroying seedlings.

The chemical protectants were effective in controlling molds and bacteria on pea seeds and seedlings, although U.S.R. No. 604 slightly excelled the other materials. Only Semesan Jr. failed to effect a slight increase in both the germination of seeds and green weight of seedlings. On the basis of green weight of seeds and seedlings Arasan and Ceresan excelled U.S.R. No. 604, Spergon, and Semesan Jr.

Arasan and Spergon permitted only 5 per cent of the pea seeds to decay in soil naturally infested with *Fusaria*, *Pythium ultimum*, and *Rhizoctonia solani*. U.S.R. No. 604 appeared to provide slightly less protection against these fungi, while Ceresan and Semesan Jr. were significantly inferior to both Arasan and Spergon.

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THE EFFECT OF ULTRAVIOLET RADIATION ON THE VIABILITY OF FUNGUS SPORES AND ON THE DEVELOPMENT OF DECAY IN SWEET CHERRIES

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(Accepted for publication August 1, 1945)

The development of fungus decay in sweet cherries during their transportation and marketing constitutes one of the most serious problems that confront the sweet-cherry industry. A number of conditions contribute to the incidence of rot, and frequently the most important factor predisposing cherries to infection is the cracking of the skin by rains which occur as the fruit approaches maturity (11). Practices that are frequently employed to control decay in sweet cherries include (*1*) the pre-harvest application of fungicidal sprays or dusts, (*2*) careful handling to avoid unnecessary injuries, (*3*) sorting to remove damaged and infected fruit, (*4*) prompt storage under refrigeration, and (*5*) the use of carbon dioxide gas as a supplement to transit refrigeration. These methods are only partially effective, and the sweet-cherry industry is constantly looking for new and more effective means of reducing its losses from decay.

During the 1944 season a few packers of sweet cherries in the Pacific Northwest installed apparatus of the latest type for irradiating the fruit with ultraviolet light, as a means of controlling decay. In the absence of factual information either on the injuriousness of ultraviolet radiation to sweet cherries or on its effectiveness in controlling decay, a series of tests was conducted, the results of which are herein reported.

LITERATURE REVIEW

The lethality of certain wave lengths of ultraviolet light to bacteria and fungi has long been recognized, and in recent years attempts have been made to utilize radiation of this type for the commercial control of undesirable micro-organisms. Fulton and Coblentz (9), using a quartz mercury-tungsten lamp, tested the killing effect of ultraviolet radiation on the spores of 27 species of fungi and also determined its efficacy in the control of fungus decay in citrus fruits. Although the rays were able to destroy a high percentage of the spores on the surface of oranges, the final amounts of rot during a simulated marketing period were very much the same in the treated and the untreated lots.

In recent years improved ultraviolet germicidal lamps have been developed which restrict most of the radiation to a wave length of 2537 A.U. (3). Rays of this type are highly effective in the destruction of bacteria and fungi, and numerous applications of germicidal lamps for the commercial control of contamination by micro-organisms have been made. They have

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been employed in the baking industry (16), in cheese manufacturing and meat storage (8), and recently in the manufacture of penicillin (12). Garrett and Arnold (10) found that 2537 A.U. radiation offered promise in the sterilization of dairy equipment, and Kligman (14) pointed out its usefulness in the laboratory.

There apparently have been few successful applications of ultraviolet light for the control of bacteria and molds in fresh fruits and vegetables, although Ewell (8) has stated that "Losses in carload shipments of celery, etc., have been greatly reduced by sterilizing the surface water used for washing by similar (Sterilamp) ultraviolet radiation." De Ong (4) has suggested the use of these rays for the control of fruit decay, but no further reference to the application of ultraviolet light for the reduction of spoilage in fresh fruits and vegetables has been found.

The injurious effect of ultraviolet radiation on higher plants has been the subject of numerous investigations but no information on the sensitivity of cherries has been located. Fulton and Coblentz (9) reported that dosages of ultraviolet light lethal to a high percentage of spores of *Penicillium digitatum* caused no evident deleterious effects in oranges, and De Ong (4) observed no injury to apricots, plums, and berries treated with this radiation.

MATERIALS AND METHODS

The tests were conducted in a commercial sweet-cherry packing plant in which ultraviolet lamps had been installed for the express purpose of reducing decay. The apparatus was designed and installed so that, theoretically, the fruit would be freed of viable fungus spores, and thereafter, until packaged, would be protected from recontamination. A battery of eight 36-inch lamps was installed so as to irradiate a 12-foot section of the fruit conveyor. The first four lamps were 18 inches from the belt and the last four were 25 inches from it. Reflectors coated with aluminum paint directed the radiation downwards onto the fruit. The conveyor was constructed so that many of the cherries would be turned over when approximately halfway through the irradiated section, thereby enabling the light to contact directly most of the surface of the fruit. Under commercial operation the speed of the conveyor was adjusted so as to irradiate the cherries for 25 seconds. The belt extended forty-eight feet beyond the outlet of the battery of lamps so as to provide space for sorting the fruit. To lower the population of airborne spores in this section of the packing room, and thus to protect the fruit from recontamination, 12 lamps were installed approximately 7 feet from the floor on each side of the conveyor. They were equipped with reflectors to direct the rays away from the workers and the fruit.

The 30-watt lamps employed in the present installation are known commercially as "Uviarc" germicidal lamps. Practically all of the ultraviolet light transmitted by them has a wave length of 2537 A.U. A complete discussion of the specifications and characteristics of these lamps is presented by Buttolph (3).

In a study of the lethality of ultraviolet radiation to fungus spores, the following seven fungi, all of which cause decay in sweet cherries, were employed: *Alternaria sp.*, *Botrytis cinerea* Fr., *Cladosporium herbarum* (Fr.) Lk., *Penicillium expansum* Lk. emend. Thom, *Pullularia sp.*, *Rhizopus sp.*, and *Sclerotinia fructicola* (Wint.) Rehm. The spores of these organisms were washed with sterile distilled water from colonies that had developed on nutrient media (Bacto potato-dextrose agar) during an incubation period of three to four weeks at 65° to 80° F. In preparing the spore suspensions of *Alternaria* and *Penicillium*, a wetting agent (Wetsit Concentrate, at a dilution of 1 part to 1400 parts of water) was used with no evident inhibition of spore germination. However, when it was found that this material prevented germination of the conidia of *Sclerotinia fructicola* its use was discontinued. After the spore suspensions had been diluted to the desired concentration (approximately 200 spores per ml.) the surface of sterile agar plates was flooded as uniformly as possible with 1 ml. of the suspension. This is essentially the same technique as that employed by Fulton and Coblentz (9). That reliable results are obtainable by this method is shown by the work of Rentschler and Nagy (17), who found that the sensitivity to ultraviolet irradiation of bacteria in the air and on the surface of agar was the same. The seeded Petri dishes, with the lids removed, were exposed to ultraviolet radiation for periods varying from 30 seconds to 5 minutes. The lamps were 18 inches above the triplicate cultures employed for each treatment. The plates were incubated at 70°–74° F. and the number of colonies that developed in each was determined. Nonirradiated plates served as controls.

To determine the efficacy of a battery of lamps (installed 18 inches above the conveyor) in sterilizing the surface of the belt, the latter was lightly scraped after having been irradiated in a fixed position for 45 minutes. The scrapings were transferred to agar plates and the viability of the material was compared with that of similar transfers made from a portion of the conveyor that had not been exposed to ultraviolet light.

The effectiveness of the lamps installed above the sorting belt in lowering the population of viable air-borne spores was ascertained after the lamps had been in commercial operation the greater part of one day. Five Petri dishes containing sterile nutrient agar were placed on the sorting belt at approximately equal distances throughout its length. The plates were exposed for 5 minutes, and after a suitable incubation period the cultures were examined for the presence of fungus colonies. A comparable series of plates was exposed in a nonirradiated section of the room.

Two types of tests, one with artificially inoculated cherries and the other with naturally contaminated fruit, were conducted to determine the efficacy of the lamps in reducing decay. In the former test, sound Lambert cherries were disinfected by immersion in a 1 : 1000 solution of mercuric chloride, and then thoroughly rinsed in sterile tap water. After the stems had been pulled, the fruit was dipped in a suspension of spores of *Penicillium ex-*

pansum, dried, and divided into 4 lots (each consisting of three 100-fruit samples). The cherries were placed in a single layer on the conveyor belt and irradiated for 0, 0.5, 1, and 5 minutes. The conveyor was operated under simulated commercial conditions. The cherries were stored at 40° F. for 10 days and removed to 65° for 3 days (equivalent to the time normally required for shipments to reach New York City and for sale through retail channels); and they were then examined for stem-end infections. At both temperatures the relative humidity was maintained at 85 to 90 per cent.

In the tests with naturally contaminated cherries, 3 lots of fruit from different localities of north central Washington were used. The first lot consisted of Bing cherries grown near Malaga. This fruit was picked 4 days after a heavy rain and had a high percentage of skin cracks. Lot 2, from the Chelan district, comprised Lambert cherries with relatively little rain damage. Lot 3 consisted of the same variety from the Squillchuck Canyon near Wenatchee. This fruit was harvested 35 days after Lot 1 and showed no evidence of rain injury. The decayed and otherwise badly damaged cherries were removed from all lots, and the remaining fruit was typical of that being packed commercially. Each lot was thoroughly composited, stored at 50° F. for 24 to 36 hours, divided into the number of triplicate 3.5- to 5-pound samples requisite for the treatments, and exposed to ultraviolet radiation in the same manner as described for the artificially inoculated fruit. The samples, unless otherwise specified, were then stored 10 days at 40° with a relative humidity of 85 to 90 per cent. All lots were examined on removal from cold storage, and Lot 1 was inspected again after 2 days at 65°.

RESULTS

Viability of Spores Irradiated in Petri Dishes. The data in table 1 show that fungi differ markedly in resistance to the lethal action of ultraviolet radiation. Based on their reaction to this light, the organisms tested can be grouped into 3 rather distinct classes, namely, highly susceptible (*Penicillium expansum*), highly resistant (*Alternaria sp.*), and intermediate (the remaining five fungi tested). Approximately 92 per cent of the conidia of *P. expansum* were killed during an exposure of 30 seconds, and all were destroyed in 2½ minutes (Fig. 1). In the case of none of the other fungi was total destruction of the spores achieved, even during an irradiation period of 5 minutes.

A slight reduction in the number of viable spores of *Pullularia* occurred during an exposure of 30 seconds, but one or more minutes were necessary to cause a significant decrease in the germination of the spores of the 5 remaining molds. Slightly over half of the spores of *Pullularia* and *Sclerotinia* were killed in 2½ minutes, but a similar reduction in the viability of the conidia of *Cladosporium herbarum* required a treatment of 5 minutes. The spores of *Alternaria* were by far the most resistant, only a few being destroyed by the maximum exposure of 5 minutes.

The spores of *Cladosporium* and *Alternaria* are dark, whereas those of the other fungi tested are hyaline or relatively light. The resistance of dark-wall spores has been observed by other workers (9, 19), and Dimond and Duggar (6) suggest the possibility that susceptibility is influenced by such factors as pigmentation, spore size, and number of nuclei. When the

TABLE 1.—*The effect of ultraviolet radiation on the viability of the spores of several fungi that cause decay of sweet cherries*

Fungus	Irradiation period	Colonies per Petri dish	Spores killed[a]
	Minutes	*Mean number*	*Per cent*
Alternaria sp.	0.0	168.7 ± 4.91[b]	0.0
	0.5	165.7 ± 7.88	1.8
	1.0	169.7 ± 6.01	0.0
	2.5	173.0 ± 9.61	0.0
	5.0	153.0 ± 3.00	* 9.3
Botrytis cinerea	0.0	84.7 ± 4.98	0.0
	0.5	76.7 ± 2.33	9.4
	1.0	78.5 ± 3.50	7.3
	2.5	72.5 ± 4.50	14.4
	5.0	25.3 ± 2.18	* 70.1
Cladosporium herbarum	0.0	230.7 ± 2.92	0.0
	0.5	218.0 ± 7.09	5.5
	1.0	220.3 ± 3.84	* 4.5
	2.5	174.3 ± 8.09	* 24.4
	5.0	104.0 ± 2.51	* 54.9
Penicillium expansum	0.0	134.0 ± 4.16	0.0
	0.5	11.0 ± 1.00	* 91.8
	1.0	3.3 ± 1.19	* 97.5
	2.5	0.0	*100.0
	5.0	0.0	*100.0
Pullularia sp.	0.0	252.0 ± 1.73	0.0
	0.5	189.7 ± 7.06	* 24.7
	1.0	179.7 ± 4.80	* 28.7
	2.5	105.0 ± 9.64	* 58.3
	5.0	75.0 ± 3.50	* 70.2
Rhizopus sp.	0.0	25.0 ± 2.08	0.0
	0.5	20.0 ± 1.52	20.0
	1.0	19.7 ± 2.19	21.2
	2.5	4.7 ± 1.19	* 81.2
	5.0	1.0 ± 0.58	* 96.0
Sclerotinia fructicola (Monilia stage)	0.0	89.7 ± 1.44	0.0
	0.5	87.0 ± 1.73	3.0
	1.0	89.3 ± 3.38	0.4
	2.5	38.3 ± 2.03	* 57.3
	5.0	12.0 ± 2.00	* 86.6

[a] Percentages marked with an asterisk differ significantly from the controls. Odds of significance 19: 1 or greater.

[b] Standard error.

poor penetration of the ultraviolet light, and the dark color and muriform structure of the conidia of *Alternaria* are taken into consideration, it is not at all surprising that this fungus proved so resistant to irradiation.

As previously stated, the installation of ultraviolet lamps and the commercial operation of the conveyor were such that the fruit would receive irradiation for only 25 seconds. Furthermore, the conveyor was designed

so that some of the cherries would be turned over after having traversed half the distance of the irradiated section, thereby exposing some parts of the fruit for only 12½ seconds. The data in table 1 indicate that *Penicillium expansum* would be the only fungus appreciably affected by this treatment. However, since this organism is not the predominant cause of spoilage in sweet cherries, it appears doubtful that effective control of decay should be expected without greatly extending the period of irradiation.

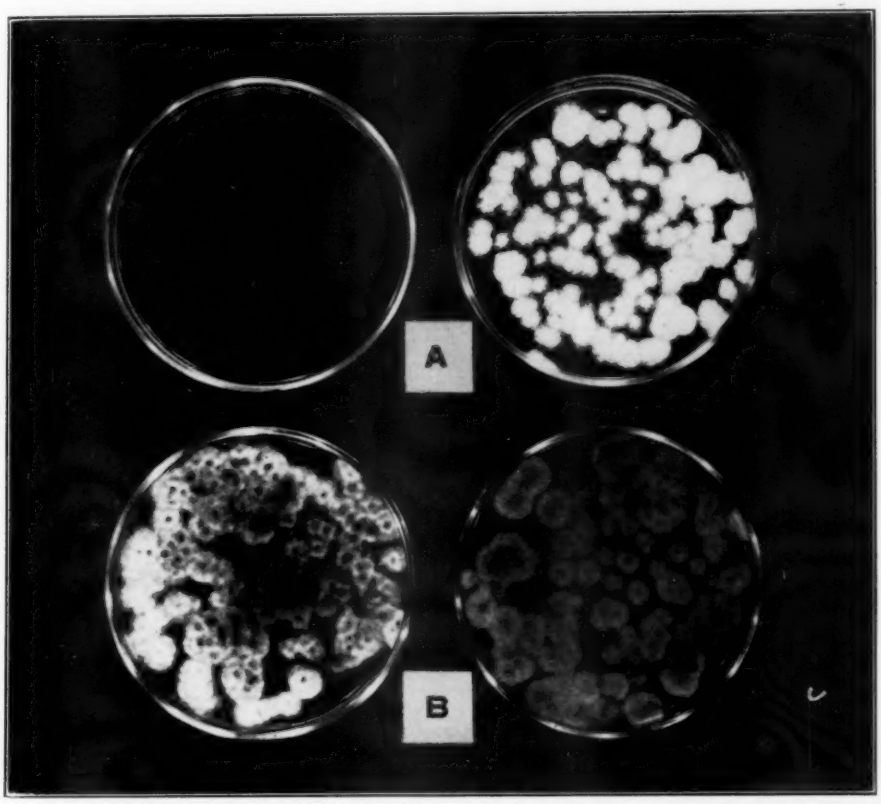

FIG. 1. Effect of ultraviolet radiation on 2 cherry molds. A, *Penicillium expansum:* plate at left irradiated 2½ minutes, nonirradiated check at right. B, *Alternaria sp.:* plate at left irradiated 5 minutes; nonirradiated check at right. Note retardation of growth and pigmentation in treated culture of *Alternaria.*

During examination of the irradiated and control cultures, it was observed that colonies developing from spores that had been exposed to ultraviolet light were smaller and less pigmented than those produced by nonirradiated spores. This condition was first observed in cultures of *Alternaria* (Fig. 1), and at that time it was thought that the apparent inhibition of growth might have been due to a killing of the more exposed cells of the spore. However, when the same effect was observed in colonies formed by the other fungi, most of which had aseptate spores, this theory had to be abandoned. It was found that the amount of inhibition was directly related

to the period of irradiation. Also, the different fungi varied considerably in the degree to which they were retarded, the colonies of *Penicillium expansum* being considerably dwarfed by an exposure of 25 seconds, whereas those of *Cladosporium herbarum* required 2½ minutes for a noticeable reduction in rate of growth. In addition to the retardation of vegetative growth, there was a concurrent lag in the formation of reproductive bodies.

Delayed spore germination resulting from the action of ultraviolet light has been observed by Dimond and Duggar (5), and Rentschler, Nagy, and Mouromseff (18) have reported a retarded development of bacterial colonies following exposure to this type of irradiation. Other workers (1, 2, 15) have shown that 2537 A.U. radiation alters agar-water gels to such an extent that they are less suitable for the development of certain bacteria, the inhibition being due, at least in part, to an increase in acidity of the medium. It

TABLE 2.—*The number of viable air-borne fungus spores on agar plates exposed in irradiated and nonirradiated sections of the packing room*[a]

Location of agar plates	Plate number	Colonies[b] per plate
Irradiated section—on sorting belt	1	13
	2	17
	3	23
	4	15
	5	22
	Average	18.0
Nonirradiated section—at elevation comparable to sorting belt	6	22
	7	19
	8	14
	9	21
	10	18
	Average	18.8

[a] Spores allowed to settle on nutrient agar plates for 5 minutes.
[b] The fungus genera obtained were principally *Penicillium*, some *Alternaria*, *Cladosporium*, *Oospora*, *Pullularia*, *Stemphylium*, and a few unidentified forms.

is not known whether the retardation of colony formation observed in the present study was due to the direct action of the light on the fungus or to some alteration of the substrate. Possibly both factors were operative.

Viability of Spores on Irradiated Fruit Conveyor. Material lightly scraped from 10 positions on the surface of a portion of the fruit conveyor that had not been exposed to ultraviolet radiation yielded fungus growth in each instance when transferred to nutrient agar. Similar material from a portion of the belt that had been irradiated continuously for 45 minutes produced colonies in 7 out of 10 transfers. The fungi isolated from the irradiated section included one or more species of *Alternaria*, *Penicillium*, *Pullularia*, and *Stemphylium*, all of which are known to cause decay of sweet cherries. The same organisms, with the addition of *Cladosporium* and *Mucor*, were obtained from the nonirradiated portion of the conveyor.

This test indicates that some of the fungus spores on the treated portion of the conveyor were destroyed, but it also shows that much viable inoculum

still remained. A quantitative test to determine the number of germinable spores on the irradiated and nonirradiated portions of the belt was not made, but judging from the data of table 1, it would seem that most of the exposed spores would be killed by treatment with ultraviolet light for 45 minutes. However, the mesh of the fabric belt, juice from injured cherries, dust, and other extraneous material would tend to protect some of the spores and thus reduce the effectiveness of the treatment.

Effect of Radiation on Air-borne Spores. The data in table 2 indicate that the ultraviolet lamps installed above the sorting belt did not reduce appreciably the number of viable fungus spores being deposited in this section of the packing room. Evidently the radiation did not contact the spores for the necessary lethal period. Also, inasmuch as the upper air in only about one-fourth of the room was irradiated, it is highly probable that spores from nonirradiated areas were deposited on the sorting belt. The conidia of *Penicillium*, which previously had shown little resistance to ultraviolet irradiation (Table 1), were found to be the predominant viable type settling

TABLE 3.—*The effect of ultraviolet radiation on the development of blue-mold decay in artificially inoculated sweet cherries*[a]

Irradiation period	Av. wt. of 3 fruit samples	Decay	
Minutes	*Grams*	*Grams*	*Per cent*
0.0 (Check)	975.7	852.7	87.4
0.5	929.0	849.3	91.4
1.0	910.0	817.0	89.8
5.0	919.0	836.3	91.0

[a] Lambert cherries, with their stems removed, were dipped in a suspension of spores of *Penicillium expansum*, irradiated, and stored for 10 days at 40° F. plus 3 days at 65°.

on the sorting belt. It is obvious that the limited radiation employed would fail to protect cherries on the sorting belt from recontamination by air-borne spores; however, if the air of the entire room were irradiated some protection could logically be expected. Whether this protection would be of commercial significance has not been demonstrated in these experiments.

Blue-mold Decay in Inoculated Cherries Treated with Ultraviolet Light. As shown in table 3, it was impossible to obtain control of decay in injured cherries that had been artificially inoculated with the spores of *Penicillium expansum*. The maximum irradiation period of 5 minutes, which was 12 times as long as the commercial exposure, failed to effect any reduction in the amount of rot. It is recognized that conditions were extremely favorable for infection and for protection of the spores from the radiation; however, openings resulting from the accidental removal of stems constitute one of the important avenues of infection in sweet cherries. The spores of *P. expansum* have little resistance to ultraviolet light (Table 1), but under the conditions of this test—actually not different from severe natural conditions—the radiation in lethal dosage evidently failed to reach the spores. These results emphasize the fact, evidently largely overlooked by those pro-

moting the use of ultraviolet light for the control of fruit decay, that the destruction of exposed fungus spores in Petri dishes is a distinctly different problem from their destruction in favorable infection courts in plant tissue.

Effect of Irradiation and Storage Temperature on the Decay of Naturally Inoculated Cherries. In tests made with 3 lots of sweet cherry fruit that differed markedly in their susceptibility to infection, ultraviolet radiation was totally ineffective in reducing decay (Table 4). Negative results were obtained when cherries were exposed to the light for 10 minutes, a period

TABLE 4.—*The effect of ultraviolet irradiation and storage temperature on the decay of naturally inoculated sweet cherries*

Test fruit	Irradiation period	Storage		Mean decay[a]	
		Temperature	Period	At removal from storage	After 2 additional days at 65° F.
		°F.	*Days*	*Per cent*	*Per cent*
Lot 1: Bing cherries severely damaged by rain	None	40	11	44.0 ± 1.74[b]	54.9 ± 2.78[b]
	25 seconds	40	11	46.9 ± 1.58	55.2 ± 2.30
	2.5 minutes	40	11	46.2 ± 0.80	53.3 ± 1.16
	5.0 minutes	40	11	41.7 ± 1.34	51.7 ± 2.06
	10.0 minutes	40	11	42.7 ± 2.07	52.4 ± 2.16
Lot 2: Lambert cherries very slightly damaged by rain	None	40	10	4.4 ± 0.55	.....
	None	50	5	* 7.2 ± 0.36	.....
		40	5		.....
	25 seconds	40	10	6.6 ± 1.50	.....
	5 minutes	40	10	5.1 ± 0.84	.....
Lot 3: Lambert cherries of relatively poor quality but without rain injury	None	40	10	21.0 ± 1.91	.....
	None	50	7	*33.6 ± 0.95	.....
		40	3		.....
	25 seconds	40	10	19.0 ± 0.76	.....
	5 minutes	40	10	20.4 ± 0.54	.....

[a] Percentages marked with an asterisk differ significantly from the checks. Odds of significance 19: 1 or greater.

[b] Standard error.

24 times as long as that used commercially. Lot 1 had about the maximum amount of rain-damaged fruit that ordinarily would be tolerated in commercial handling, and the decay that developed in this lot emphasizes the hazard connected with the shipment to eastern markets of this class of cherries. The fruit of Lot 2 was of exceptionally fine quality, and had a cull grade of only 2 per cent when received from the grower. If the percentages of decay appear unduly high, it is because injured fruit with mycelium visible to the naked eye on the exposed tissue, but without evident penetration of the sound flesh, was classed as decayed.

The importance of proper lowering of transit temperature in the control of brown rot and blue-mold rot of sweet cherries has previously been discussed (7). The stimulatory effect of relatively high storage temperature

(50° F.) on the development of natural rots is shown in table 4 (lots 2 and 3). Irradiation of the fruit up to 24 times as long as is commercially practiced failed to reduce decay, whereas a difference in temperature of 10 degrees F. for only 5 days had a significant influence on the amount of spoilage. Ultraviolet radiation, as used in these studies, appears to offer little if any promise for the control of decay in sweet cherries, whereas the maintenance of proper storage and transit temperatures definitely reduces such losses.

One of the questions raised by some of the handlers of sweet cherries prior to the installation of ultraviolet-ray equipment was the possible injurious effect of this radiation on the fruit. Careful examination of the irradiated cherries and their stems failed to show any evidence of injury, even following the maximum periods of exposure. Similar results with oranges were obtained by Fulton and Coblentz (9).

DISCUSSION

It has been found that the spores of the more common fungi causing decay in sweet cherries can be destroyed by ultraviolet radiation if the requirements for proper wave length, intensity, and period of exposure are fulfilled. With information such as this at their disposal and with the knowledge that infection does not occur unless spores come in contact with fruit, some individuals have assumed that irradiation of contaminated plant material would largely prevent the development of decay. The fallacy of such reasoning has been shown in the present experiment by irradiating artificially and naturally contaminated cherries for periods considerably longer than necessary to kill most of the exposed fungus spores. It is the spores that have become lodged in ruptures in the skin of the fruit that are largely responsible for decay, and these are the ones that ultraviolet light seems incapable of reaching. Evidently most of the injury and contamination of the fruit occurs prior to the time it is unloaded at the packing plant, and the subsequent partial disinfecting of the cherries by irradiation has no appreciable effect on the development of decay.

Apples become heavily contaminated with fungus spores in the orchard (13) and there is little doubt that cherries are similarly inoculated. This condition is entirely different from that in bakeries and many other food-processing plants in which the original product is essentially free from spores or bacteria, and the problem is to protect it from contamination. Here the use of ultraviolet radiation to lower the population of spores in the air and on equipment would be expected to prove beneficial.

As a means of sterilizing the fruit conveyor, ultraviolet light proved partially effective, and if the upper air in the entire packing room had been continuously irradiated there is little doubt that the population of air-borne spores would have been reduced. However, from the results obtained in the present experiment it appears doubtful that either factor would appreciably affect the amount of decay developing in sweet cherries.

SUMMARY

The effect of ultraviolet radiation (wave length 2537 A.U.) on the spores of 7 fungi that cause decay of sweet cherries, and on the development of decay in this fruit was determined.

The fungi showed extreme variation in the susceptibility of their spores to the lethal action of the radiation. Most of the spores of *Penicillium expansum* were killed in 30 seconds, whereas only a few of the conidia of a species of *Alternaria* were destroyed after an exposure of 5 minutes. Spores with dark-color walls showed greater resistance than those with light-color walls. Colony formation from irradiated spores that were not killed was noticeably retarded.

The viable spore-load on the fruit conveyor in a commercial sweet-cherry packing plant was reduced by exposure to ultraviolet light, but the number of living air-borne spores in an irradiated section of the packing room did not differ significantly from the number found in a portion of the room that received no ultraviolet treatment.

Blue-mold decay in artificially injured and contaminated sweet cherries was not reduced by exposure to ultraviolet light.

Natural decay of sweet cherries was not controlled by ultraviolet radiation, even though the period of exposure was extended up to 24 times that employed commercially. As used in these studies, ultraviolet light appears to offer no promise for the control of decay, whereas the maintenance of proper storage and transit temperatures definitely reduces losses from decay.

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FIELD STRAINS OF TOBACCO-MOSAIC VIRUS

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(Accepted for publication August 10, 1945)

In the laboratory many strains of the tobacco-mosaic virus may be isolated from a given field strain if the virus of the field strain is "plated out" on a necrotic-spotting variety of tobacco or certain other species of *Nicotiana*, and transfers are made from individual spots to susceptible plants (2, 3, 6, 7). These isolants may be less aggressive than the original field strain and be outgrown by the latter when both are mixed in a given plant. Some of these isolants might be purely laboratory strains which could not survive in the field. As a result, perhaps, of such studies as these, some laboratory workers not too familiar with the tobacco mosaic disease in the field, have taken the view that there is but one strain, or at best only a few strains, of the tobacco mosaic virus in the field. For example, one writer (1) states that by various isolation technics, "literally hundreds of different strains can be obtained"; yet, he states, "when one goes into the field to obtain tobacco mosaic virus, one always finds the same strain in the tobacco fields. Why? The strain is nature's fittest." This not uncommon view has led to a great deal of confusion in the literature.

Several papers have been published, in which the virus used has been identified by the term Tobacco Virus 1 or Nicotiana Virus 1, inferring that this description is sufficient to identify the strain in the future. By definition Tobacco Virus 1 and Nicotiana Virus 1 include most of the field strains of the virus studied by virus workers from the time of Mayer to the present (5, 8). There is no way of knowing how many distinct strains of the tobacco mosaic virus have been studied during this period. When inoculations are made from cigars, cigarettes, or other dried tobacco, isolated yellow spots often develop on the first-invaded leaves, indicating that yellow strains are abundant in nature. Yellow and white strains are also frequently found in solanaceous weeds growing in old tobacco-growing areas. More conclusive proof that numerous strains are established in nature is furnished if collections are made from tobacco and solanaceous weeds in old tobacco-growing areas where tobacco is rotated with grass, thus allowing the perennial hosts to persist. Under these conditions the perennial solanaceous weeds become infected and act as reservoirs of virus for years.

Tobacco plants also become infected from dried mosaic tobacco of previous crops, from the hands of workers. Infection of seedling plants by individual virus particles may be frequent, and mutant strains, if present, could become established in pure culture in individual plants. If the strain happened to be nonaggressive it might, in the life of a vigorously growing tobacco plant, produce a more aggressive mutant and be replaced by it or it might become localized and lost. If aggressive, the new strain might be spread by the usual methods in the field and become a well-established field

strain with no chance of competition from less vigorous strains. In old tobacco-growing areas it is not unusual to find predominent strains on nearly every farm, strains which often differ from farm to farm. On large farms, where much transient labor is used, many strains of the virus are found. It is thus possible for numerous mutant strains to become established and maintain themselves in nature. There is accumulating evidence that numerous field strains of the tobacco mosaic virus occur. In 1928 Valleau and Johnson (10) mentioned 4 distinct field strains, in 1930 Johnson (4) described 7, and in 1936 Valleau (9) described 18 distinct field strains in a key which indicated the complexity of the tobacco mosaic problem in the field.

The purpose of this paper is to present additional evidence that numerous strains of the tobacco mosaic virus occur in nature and that these strains produce a complex of symptoms in tobacco, variable both with tobacco variety and environment, which makes it nearly impossible to describe a virus strain with sufficient clarity so that it can be identified with a strain described by another worker.

EXPERIMENTAL EVIDENCE

Four necrotic-spotting (N′N′) tobacco varieties (11), Kelly and Judy (Burley), Kentucky 120 and 129 (dark fire-cured), in the greenhouse, were inoculated in duplicate with dried material of 54 field collections of tobacco mosaic virus. Inoculations were in triplicate, on field-grown plants of these varieties (except Ky. 120), with the 54 virus strains. Inoculations were also made to a non-necrotic-spotting variety (Ky. 16 n′n′) but many of the viruses appeared so much alike on this variety that it was not considered a good test plant for showing differences in strains. The dried samples used in these tests were collected during the past 15 years from naturally affected tobacco plants from Kentucky fields, with the following exceptions: one each from tobacco from Florida and North Carolina; 3 from garden pepper, and 2 from *Physalis sp.* Included in these tests was a strain (No. 52 in table 1) of tobacco mosaic virus from the 1882 burley crop. Many more field strains of tobacco mosaic, apparently different from those used in this study, have been collected from tobacco plants and weeds, indicating that the number of tobacco-mosaic strains of natural occurrence is almost unlimited.

For convenience of description, the tobacco-mosaic strains are grouped in table 1, on the basis of symptoms caused in the necrotic-spotting varieties of tobacco. In the greenhouse the 54 tobacco-mosaic strains could be placed roughly in 19 groups; however, no two strains within a group were identical. Affected plants within a group had the same general type of symptoms but there were differences in color and distribution of mottling, degree of distortion, size and type of local spots, and degree of dwarfing, that were observable only by direct comparison.

In the field, symptom differences were greater and more varied. A minimum of 33 groups was required to classify them. For example, in the greenhouse, collection 9 caused slight dwarfing and distortion and produced yellow

ring-like patterns in all varieties tested, while in the field, the symptoms were almost identical with those caused by "pure white" mosaic (collection 1).

TABLE 1.—*Symptoms*[a] *caused by field strains of tobacco mosaic virus in necrotic-spotting (N'N') varieties of tobacco*

Laboratory number of virus	Type of localization		Type of mottling		Degree of dwarfing		Degree of distortion		Type of necrosis		Group number	
	GH	*F*	*GH*	*F*	*GH*	*F*	*GH*	*F*	*GH*	*F*	*GH*	*F*
1	N	N	O	W	O	+	O	+++	O	O	1	1
40	N	N	Y	G	+	+	+	++	O	O	2	2
30	N	N	Y	G	+	+	+	++	O	O	2	3
51	N	N	Y	YR	++	+	+	+	O	O	3	4
7, 24	N	N	Y	YR	+++	+	+++	+++	St	O	4	5
10, 18	N	N	Y	Y	+++	+	+++	+++	St	O	4	6
32, 52	N	N	Y	G	+++	+	+++	+++	St	O	4	7
28	N	C	Y	G	+++	+	+++	+++	St	O	4	8
13, 14	C	C	YR	Y	+	+	+	+++	O	O	5	9
29, 39	C	O	YR	Y	+	++	+	++	O	O	5	10
36	C	O	Y	G	+	+	+	+	O	S	6	11
43	C	O	Y	G	+	+	++	+	O	BS	7	12
38	C	O	G	G	+	+	++	++	O	B	8	13
16	C	C	Y	G	+++	+	+, E	++	O	B	9	14
22	C	C	YR	Y	+	+	+	+++	B	S	10	15
49	C	O	Y	YR	+++	+	+, E	+	B	B	11	16
46	C	C	Y	Y	+++	++	+, E	+++	B	B	11	17
34	C	C	Y	Y	+++	+	+, E	+++	B	O	11	18
9	O	N	YR	W	+	+	+	+++	O	O	12	1
12, 15, 23, 33, 41	O	C	YR	Y	+	+	+	+++	O	O	12	18
42	O	C	Y	G	+	+	+	+++	O	O	13	8
4	O	O	G	YR	++	+	+	+	O	O	14	19
16	O	O	G	YR	++	++	+	++	O	O	14	20
19	O	C	G	Y	++	+	+	+	O	O	14	21
21, 26, 27, 53	O	O	G	YR	++	+	+	++	O	O	14	22
37	O	O	G	G	++	+	+	+	O	B	14	23
6	O	O	Y	Y	++	++	++	+++	O	B	15	24
8, 11	O	O	Y	Y	++	++	++	+++	O	BS	15	25
25	O	O	Y	Y	++	++	++	+++	O	S	15	26
35	O	C	Y	Y	++	++	++	+++	O	O	15	27
20	O	C	G	G	++	++	+	+++	O	BS	16	28
45	O	C	G	G	++	+	+	+++	O	S	16	29
2, 3	O	O	G	G	+++	++	+++, E	+++	O	O	17	30
5, 31, 54	O	C	G	Y	+++	++	+++, E	++	O	S	17	31
44	O	C	Y	G	++	++	+	+++	B	BS	18	28
48	O	O	Y	G	+	+++	+, E	+++	B	S	19	32
47	O	O	Y	G	+	++	+, E	+++	B	BS	19	33
50	O	C	Y	G	+	++	+, E	+++	B	BS	19	28

[a] Symptoms in column *GH* occurred in the greenhouse, those in column *F* in the field.
O indicates no symptoms.
N and C denote local necrotic or chlorotic spots.
Y, W, and G denote yellow, white, or green mottling.
YR denotes yellow rings.
B and BS denote burn and burn + necrotic speck spots in leaves.
S denotes necrotic speck spots in leaves.
St denotes necrotic streak of leaf veins and mid vein.
E denotes enation.
+, ++, and +++ denote slight, moderate, and severe distortion or dwarfing.

"Pure white" mosaic did not become systemic in greenhouse tobacco plants, but in the field this strain became systemic and caused marked distortion.

All viruses of greenhouse group 4 caused marked dwarfing and necrotic streaking of veins and midveins of invaded leaves. None of these caused streak in the field, in this test, and because of dissimilarity of symptoms, they were placed in 4 distinct groups. Other striking shifts in grouping in the field are observable in table 1. For example, the viruses of greenhouse group 14 formed 5 distinct groups in the field.

The descriptions in table 1 are not specific enough for identification of the viruses. They are given only to show that many strains of tobacco mosaic exist in nature. This study indicates that it is unsafe to assume that one is working with the same tobacco virus strain used by another worker merely because it was obtained from an infected plant in the field and compares fairly closely with published descriptions of symptoms on a single variety of tobacco and under one set of environmental conditions, particularly if the descriptions are based on the use of a non-necrotic-spotting variety (n′n′) of tobacco. Two viruses may appear identical on a non-necrotic-spotting variety, whereas on a necrotic-spotting variety one may produce necrotic spots and streak, while the other may cause only mottle. The terms Tobacco Virus 1, common field strain, or wild strain of tobacco mosaic virus, if used to designate a specific virus commonly distributed in nature, are misleading.

SUMMARY

Fifty-four dried samples of tobacco, affected with tobacco mosaic, collected during 15 years in Kentucky tobacco fields and elsewhere, were used to inoculate necrotic-spotting varieties of *Nicotiana tabacum* in the greenhouse and field. The symptoms caused by these samples varied greatly both in the greenhouse and field. There were all shades of mottling from pure white to dark green, various degrees of distortion and dwarfing, various types and sizes of chlorotic and necrotic local spots, and various degrees of burn. No two collections caused identical symptoms, either in the greenhouse or field, although symptoms on replicate plants were identical. On a basis of similarity of symptoms and for convenience of arrangement in tabular form, the collections were placed in a minimum of 19 groups in the greenhouse. Thirty-three groups were the minimum into which the 54 strains could be placed in the field tests.

Most of these tobacco-mosaic-virus collections, if on n′n′ tobacco plants could, by definition, be considered Tobacco Virus 1. For this reason it is suggested that the use of the term, Tobacco Virus 1, in the sense that it describes a specific entity, is not justified nor is it desirable to use the terms, common field tobacco-mosaic virus, or wild-type tobacco-mosaic virus to indicate a specific entity, for the same reason.

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LEAFHOPPER TRANSMISSION OF THE VIRUS CAUSING PIERCE'S DISEASE OF GRAPE AND DWARF OF ALFALFA

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(Accepted for publication August 13, 1945)

This paper presents methods and data mentioned in previously published abstracts on the finding of leafhopper vectors of the virus causing alfalfa dwarf (6), the virus causing Pierce's disease of grape (3), and the association of the two diseases in the field (4). In addition, it describes the methods used in intertransmission experiments between alfalfa and grape, and the data obtained.

LEAFHOPPER TRANSMISSION OF THE ALFALFA DWARF VIRUS

Weimer (8, 9, 10, 11) determined that the alfalfa dwarf disease was caused by a virus transmissible by grafting, that it spread naturally in experimental plots in the field, and that it was apparently insect-transmitted. He (11) tested some insects, but did not report any transmission.

The distribution of diseased alfalfa plants in the fields follows, as Weimer remarks, a pattern indicative of insect transmission. During this investigation, in new fields planted adjacent to severely infested fields the disease could be found in the new plantings on the edge nearest the older plantings within 8 to 10 months after the new ones had become established. This occurred whether the new field immediately adjoined the old one or was separated from it by a road or by other crops so that each field was culturally treated as a separate unit. Virus-disease movement of this type points to an insect as the vector.

As mentioned in an abstract (6), the results of field caging trials in 1940 indicated that the virus was transmitted by insects. Two muslin-covered cages were constructed on land not previously seeded to alfalfa, with each cage covering 100 square feet. Alfalfa of the California Common variety, seeded under each cage, was allowed to grow for about three months. Then, collections of most insects commonly found in diseased fields were introduced into cage A, and insects were excluded from cage B as far as possible. Six months after the insects were introduced, 63 per cent of the plants in cage A had the dwarf disease as compared with 1 per cent in cage B from which insects were excluded. Five months later, less than 15 per cent of the original stand remained alive in the insect-infested cage A, and 44 per cent of these showed typical dwarf symptoms; in the insect-free cage, B, approximately 100 per cent of the original stand remained, and less than 2 per cent

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of these were diseased. The small percentage of disease in the insect-free cage B, was apparently the result of transmission by insects found to have accidentally gained entrance into the cage.

Transmission tests were made with over twenty species of insects, including aphids, beetles, leafhoppers, treehoppers, and thrips. These, except the thrips, were collected in diseased alfalfa fields and caged for 24 hours on dwarf-diseased plants before being caged on healthy plants. The thrips were collected on diseased alfalfa plants and transferred directly to healthy plants. Four months after the date of caging, the plants were dug and examined. The only transmissions occurred in two of the three trials with *Draeculacephala minerva* Ball. A later trial with 100 individuals of this species, caged on dwarf-diseased alfalfa plants and subsequently transferred in one group to 30 healthy plants in a single cage, resulted in infection of

TABLE 1.—*Natural infectivity of four species of leafhoppers collected in their natural habitats and transmission of alfalfa-dwarf virus from diseased to healthy alfalfa plants by these leafhoppers*

Species of leafhopper	No. of collections	Lots of insects tested[a]		No. of plants inoculated	No. of transmissions
		No.	State of natural infectivity[b]		
Draeculacephala minerva	15	2	Positive	57	55
		45	Negative	91	60
Carneocephala fulgida	5	0	Positive	0	0
		5	Negative	14	6
Helochara delta	3	0	Positive	0	0
		10	Negative	26	12
Neokolla circellata	5	14	Positive	93	69
		2	Negative	2	2

[a] The number of hoppers in each lot tested varied from 20 to 40 individuals.
[b] Positive indicates that these lots of leafhoppers were carrying the dwarf virus when collected; Negative, that they were not carrying the dwarf virus when collected.

93 per cent of the plants. A similar test with a closely related leafhopper, *Carneocephala fulgida* Nott., resulted in 19.4 per cent infection.

During 1941 through 1943, controlled insectary inoculations were made testing several species of insects collected in affected areas and in areas widely separated from any diseased alfalfa plantings. These collections were separated into lots of 20 to 40 individuals. Usually, before being used for transmission tests, each lot was fed on a series of healthy alfalfa plants to determine whether any insects in that lot were naturally viruliferous. After incubation of the plants used for these natural infectivity tests, it was possible to separate transmissions obtained by lots of leafhoppers proved naturally viruliferous from transmissions by those apparently nonviruliferous. As a rule, when the vectors were collected in areas where the virus was prevalent in alfalfa, they were naturally viruliferous. Collections in areas widely distant from dwarf-diseased alfalfa varied considerably in the percentage of lots proving to be naturally infective. Occasionally repeated collections

from an isolated population have been free of the virus. This method of obtaining nonviruliferous leafhoppers was necessary because of inability to rear sufficient populations in the insectary.

Table 1 shows the results of natural infectivity and dwarf-virus transmission tests with four species of leafhoppers. Because an attempt was made to collect the vectors in areas known to be relatively free of the virus, the majority of lots of three of the species were not naturally infective. The collections of *Neokolla* (*Cicadella*) *circellata* (Baker) were, however, usually so.

With *Draeculacephala minerva* Ball a total of 47 lots, 45 of which were negative in the natural infectivity tests, were used in the transmission trials. After feeding on diseased alfalfa, the two lots shown to be naturally infective were fed on 57 healthy alfalfa plants, with resultant transmission of the virus to 55 of these. The naturally noninfective lots, after feeding on diseased alfalfa, infected 60 of 91 plants inoculated. All lots of *Carneocephala fulgida* Nott. tested were naturally noninfective, and of 14 plants inoculated 6 were infected. With *Helochara delta* Oman[5] the ten lots tested were naturally noninfective, and 12 of 26 inoculated plants were infected. Fourteen of the 16 lots of *Neokolla circellata* (Baker) tested were naturally infective and infected 69 of the 93 plants inoculated. With the two noninfective lots, only two plants were inoculated; both developed the disease.

Numerous transmission trials were made with other collections for some of which the natural infectivity was not tested. The results are included in table 6.

LEAFHOPPER TRANSMISSION OF THE VIRUS CAUSING PIERCE'S DISEASE OF GRAPE

Pierce's disease of the grapevine (3, 5), recognized in California as early as 1884, was described by Newton B. Pierce in 1892 under the name "California vine disease" (7). It subsided around 1910, but became noticeably active again in grapevines about 1934 (5). In 1938 it was shown to be transmissible by grafting (2), and thereafter it was renamed Pierce's disease (3). The discovery that Pierce's disease was transmissible by grafting and was probably caused by a virus led to a search for possible means of spread. The pattern of diseased-grapevine distribution suggested some insect as a vector. The distribution followed two general patterns: first, diseased vines irregularly scattered through the vineyard; second, diseased vines concentrated in relatively small areas. Both types of distribution were found in most vineyard districts of the State on varied soil types and under different cultural practices. Localized spots of diseased vines were often found in portions of vineyards where water had been allowed to stand after irrigation and in other places where grasses continued to flourish, such as margins near irrigated pastures and alfalfa fields. These places usually supported numerous insects and the presence of diseased vines indicated that they included possible vectors.

[5] Determined by P. W. Oman, Bureau of Entomology and Plant Quarantine, Division of Insect Identification, Washington, D. C.

Further evidence of insect transmission was obtained during 1940 in vineyards where insect light traps fitted with gas-discharge tubes and electrocuting grids had been installed. The traps were operated after dark for control of the grape leaf-folder. In a vineyard where an experiment with different light traps was being conducted,[6] one 10-acre block of vines fitted with four 250-watt blue mercury-vapor-tube light traps had diseased grape vines around each trap, as shown in figure 1. Also, according to results of insect-net sweepings taken in this block of the vineyard, there was a higher population of several species of leafhoppers on the grass and weed

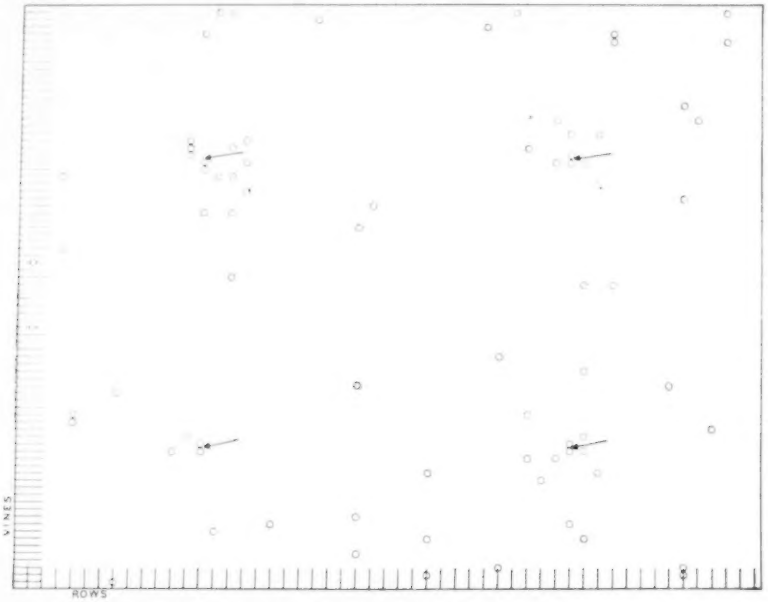

FIG. 1. Concentration of vines affected with Pierce's disease near the lights in a 10-acre vineyard in which were installed four 250-watt blue mercury-vapor-tube light traps fitted with electrocuting grids. The circles indicate the position of the diseased vines present in the vineyard in the spring of 1940. The arrows indicate the location of the light traps.

cover beneath the four light traps than elsewhere. The information obtained from these traps narrowed the scope of insects to be tested as possible vectors.

The general plan for discovering a vector of Pierce's disease was to test, wherever they could be found, all insects that might possibly transmit the virus. Preliminary tests were made on young vines growing in a plot located in a generally infested area. Large numbers of control vines were maintained, interspersed among the test vines to indicate possible natural spread. The insects were collected with hand nets, segregated, and placed in cheesecloth bags with cellophane windows. These bags were placed over foliage of diseased vines, whence they were removed later, after 1 to 3 days' feeding of the insects. The bags containing the insects were then placed over foliage

[6] This experiment with light traps was being conducted by J. K. Ellsworth, Division of Entomology and Parasitology, University of California.

of healthy test vines, where they were kept, usually, until the insects were dead.

During 1939, as previously mentioned (3), 54 species of insects were tested on 94 separate grapevines. Ten vines developed symptoms of Pierce's disease, but only one out of 215 controls did so. During 1940 a total of 60 species, including some of those previously tried, were similarly tested. Six out of 21 vines caged with *Draeculacephala minerva* Ball and 3 vines out of 19 caged with *Carneocephala fulgida* Nott. developed symptoms. Only 6 out of 506 control vines developed the disease. The symptoms were the same as on vines grafted with diseased wood.

During 1941 through 1943, tests were conducted in an insectary. All trials were made in insect-proof cages in a screen house or in a greenhouse.

TABLE 2.—*Natural infectivity of four species of leafhoppers collected in their natural habitats and transmission of Pierce's disease virus from diseased to healthy grape by these leafhoppers*

Species of leafhopper	No. of collections	Lots of insects tested[a]		No. of plants inoculated	No. of transmissions
		No.	State of natural infectivity[b]		
Draeculacephala minerva	6	2	Positive	10	2
		23	Negative	40	16
Carneocephala fulgida	3	0	Positive	0	0
		5	Negative	10	4
Helochara delta	2	0	Positive	0	0
		4	Negative	11	7
Neokolla circellata	10	28	Positive	46	37
		8	Negative	8	4

[a] The number of leafhoppers in each lot tested varied from 20 to 40 individuals.

[b] Positive indicates that these lots of leafhoppers were carrying the virus of Pierce's disease when collected; Negative, that they were not carrying the virus of Pierce's disease when collected.

The grapevines used were rooted cuttings of the varieties Emperor, Malaga, Molinera, and Thompson Seedless, growing in greenhouse pots.

Leafhoppers from various locations were brought into the insectary and segregated into small lots. Many lots were first caged in succession on 2 to 3 healthy plants and held on each plant from 2 to 5 days to test them for natural carriers of the virus (state of natural infectivity). Each lot was then caged on a vine that had Pierce's disease and was held there 1 to 3 days. The lot of insects was then transferred in succession at 2- or 3-day intervals to one or more healthy vines for transmission trials. Table 2 gives the results of these trials with four species of leafhoppers: *Draeculacephala minerva* Ball, *Carneocephala fulgida* Nott., *Helochara delta* Oman, *Neokolla circellata* (Baker). Leafhoppers in 2 out of 25 lots of *D. minerva* and 28 out of 36 lots of *N. circellata* carried the virus of Pierce's disease naturally when brought into the insectary for transmission tests. Previously noninfective lots of four species of the leafhoppers transmitted the virus from diseased to

healthy grapevines. Additional transmission tests with several lots of leafhoppers of the four species mentioned above were made without first testing the entire collection for natural carriers. The results of these tests, compiled with others, are presented in table 6. In addition to the above named leafhoppers, Frazier (1) recently reported 6 other species of leafhoppers as vectors of the Pierce's disease virus.

THE ASSOCIATION OF ALFALFA DWARF AND PIERCE'S DISEASE

The relation of alfalfa fields to the prevalence of Pierce's disease in grapes was noticed in infested districts where the two crops adjoined each other (4). Diseased vines were usually much more prevalent in the portion of vineyards nearest the alfalfa fields. In some instances, 100 per cent of the vines were infected in the first 4 to 6 rows adjacent to alfalfa. A typical

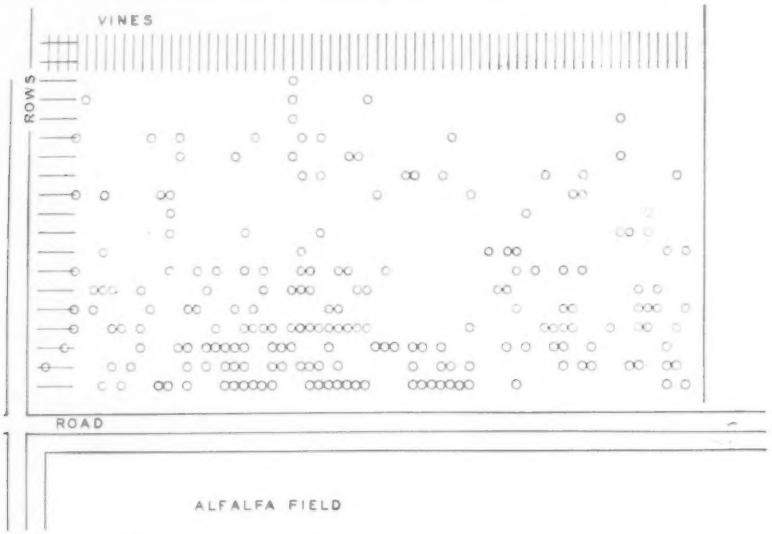

FIG. 2. Prevalence of vines manifesting Pierce's disease in a portion of a vineyard adjacent to an alfalfa field infested with dwarf. Very few diseased vines were found in the rest of the vineyard. Each circle indicates a diseased vine.

example is illustrated in figure 2, showing an alfalfa field, bordered on the north side by vineyard. The effect of the alfalfa was evident in the abundance of Pierce's disease in the first 12 rows of grapes adjacent to it.

The association suggested that alfalfa was harboring a vector of the grape disease, or that the alfalfa was an alternate host of the virus, or that it functioned in both capacities. Such alfalfa plantings usually contained a high percentage of dwarf-infected plants.

Furthermore, in vineyard districts where some alfalfa was planted and where Pierce's disease occurred, the alfalfa fields were usually infested with dwarf. The converse was also true. In certain districts the two crops existed separately, in some places with and in other places without their respective diseases.

The first efforts to connect the two diseases with the same causal agent were by means of mechanical inoculation. Neither dwarf of alfalfa nor Pierce's disease of grape was successfully transmitted by various means of juice inoculation. As previously reported, however, one can transmit dwarf to alfalfa and Pierce's disease to grape by grafting pieces of diseased plants on healthy plants. Pieces of roots from diseased alfalfa were therefore inserted into roots of healthy alfalfa and into stems of healthy grape; and, conversely, pieces of roots from diseased grape were inserted into roots of healthy alfalfa and healthy grape.

In these tests the following results were obtained: from diseased alfalfa to healthy alfalfa, dwarf was transmitted in 7 out of 10 trials; from diseased alfalfa to healthy grape there were no apparent transmissions; from diseased grape to healthy grape, Pierce's disease was transmitted in 37 out of 46 trials; and from diseased grape to healthy alfalfa, 12 out of 35 alfalfa plants showed dwarf symptoms after 6 months' incubation. This indicated the identity of the viruses.

INTERTRANSMISSION TESTS BETWEEN GRAPE AND ALFALFA PLANTS WITH LEAFHOPPER VECTORS

The discovery that dwarf of alfalfa and Pierce's disease of grape could be transmitted by the same vectors led to intertransmission experiments with the four species of leafhoppers. The plan of the following intertransmission experiments was to attempt to transfer the virus causing Pierce's disease of grape to alfalfa, and the virus causing dwarf of alfalfa to grape by means of the leafhoppers.

Leafhoppers of the species known to be vectors were collected from various natural populations, some from districts apparently free of either disease and others from districts known to be infested. The natural infectivity of some of these lots of leafhoppers used in intertransmission experiments was tested by caging a single lot of 20 to 50 leafhoppers on each of 2 to 4 healthy plants of both alfalfa and grape in succession. Each lot of insects was held on each individual plant for 1 to 3 days. The same individual lot was then caged on a diseased plant, either grape or alfalfa, and then subsequently transferred in successive order to healthy alfalfa and healthy grape plants or vice versa. The leafhoppers were retained on each plant from 1 to 6 days. The state of natural infectivity was not determined for all lots of field-collected leafhoppers. Only random sample lots of leafhoppers were taken from some lots and tested for the presence of natural carriers of the virus. Usually the remainder of the collection was divided into lots, and each lot caged directly on diseased plants, after which it was transferred to healthy ones. All test plants were then held in large screened cages or isolated in a greenhouse for incubation.

Some lots of field-collected leafhoppers proved to be naturally infective (viruliferous); that is, as collected in nature they contained individuals that carried a virus or viruses that would infect alfalfa and grape. The alfalfa

TABLE 3.—*Results of successive individual plant transfers of a single field collection of leafhoppers (N. circellata) which was naturally viruliferous when collected*

Plants on which leafhoppers were caged	No. of leafhoppers	No. of days on plant	Condition of plant after incubation
Alfalfa	25	3	Dwarf
Grape	23	2	Pierce's disease
Grape[a]	23	2	
Grape	10	2	Pierce's disease
Alfalfa	10	2	Dwarf
Grape	10	3	Pierce's disease
Alfalfa	8	2	Dwarf

[a] Plant used as source of virus in experiment; however, incubation of the test plants showed the leafhoppers to be naturally viruliferous.

plants developed symptoms identical with dwarf, and the grape, symptoms identical with Pierce's disease. Table 3 shows the results of transmissions with such a lot of leafhoppers of *Neokolla circellata*.

Other lots of leafhoppers as collected from the field were apparently virus free as far as alfalfa and grape were concerned, for they did not transmit a virus to the healthy plants used in the natural infectivity tests, but did carry one to healthy plants after having been caged on diseased grape or alfalfa. Table 4 shows the results of intertransmission tests of the virus from dwarf-diseased alfalfa to healthy alfalfa and grape with a single lot of *Draeculacephala minerva*. The 45 leafhoppers in this lot were first placed successively at two-day intervals on two healthy alfalfa plants and one healthy grape plant to test their natural infectivity. These three plants remained healthy showing that this lot of leafhoppers did not naturally carry the virus. After being fed on dwarf-diseased alfalfa, the insects were trans-

TABLE 4.—*Results of successive individual plant transfers of a single field collection of leafhoppers (Draeculacephala minerva) from alfalfa to grape with dwarf-diseased alfalfa as a source of virus*

Plants on which leafhoppers were successively caged	No. of leafhoppers	No. of days on plant	Condition of plant after incubation
Alfalfa	45	2	Healthy
Alfalfa	38	2	Healthy
Grape	34	2	Healthy
Alfalfa, dwarf[a]	34	2	
Grape	11	2	Pierce's disease
Alfalfa	9	2	Dwarf
Grape	9	2	Pierce's disease
Alfalfa	8	2	Dwarf
Grape	8	2	Pierce's disease
Alfalfa	8	3	Dwarf
Grape	8	2	Pierce's disease
Alfalfa	5	2	Dwarf
Grape	5	2	Pierce's disease
Alfalfa	5	2	Dwarf

[a] Dwarf-diseased alfalfa plant used as virus source; the first three plants were used to determine the presence or absence of viruliferous individuals in the field-collected hoppers.

ferred alternately to 5 healthy grape and 5 healthy alfalfa plants. After several months all of the grapes manifested Pierce's disease, and all the alfalfa plants showed dwarf symptoms.

Table 5 shows the results of intertransmission of the virus from 5 differ-

TABLE 5.—*Results of virus transmission tests from grape to alfalfa and alfalfa to grape with ten different field collections of leafhoppers (Draeculacephala minerva)*

Leafhopper lot No.	Plant upon which collection was successively fed	No. of leafhoppers	Days on plant	Condition of plant after incubation
1	Grape[a]	15	3	Healthy
	Pierce's disease grape[b]	7	2	.....
	Grape	4	2	Pierce's disease
	Alfalfa	4	6	Dwarf
2	Grape	15	3	Healthy
	Pierce's disease grape[b]	11	2	.....
	Grape	8	2	Pierce's disease
	Alfalfa	4	6	Dwarf
3	Grape	15	3	Healthy
	Pierce's disease grape[b]	7	2	.....
	Grape	5	2	Pierce's disease
	Alfalfa	2	6	Dwarf
4	Grape	15	3	Healthy
	Pierce's disease grape[b]	12	2	.....
	Grape	11	2	Pierce's disease
	Alfalfa	6	6	Dwarf
5	Grape	15	3	Healthy
	Pierce's disease grape[b]	9	2	.....
	Grape	8	2	Pierce's disease
	Alfalfa	4	6	Dead (cause unknown)
6	Alfalfa	15	4	Healthy
	Alfalfa, dwarf[b]	14	2	.....
	Alfalfa	12	2	Dwarf
	Grape	10	6	Pierce's disease
7	Alfalfa	15	4	Healthy
	Alfalfa, dwarf[b]	13	2	.....
	Alfalfa	11	2	Dwarf
	Grape	11	6	Pierce's disease
8	Alfalfa	15	4	Healthy
	Alfalfa, dwarf[b]	15	2	.....
	Alfalfa	14	2	Dwarf
	Grape	14	6	Pierce's disease
9	Alfalfa	15	4	Healthy
	Alfalfa, dwarf[b]	12	2	.....
	Alfalfa	9	2	Dead (cause unknown)
	Grape	5	6	Pierce's disease
10	Alfalfa	15	4	Healthy
	Alfalfa, dwarf[b]	13	2	.....
	Alfalfa	13	2	Dwarf
	Grape	12	6	Pierce's disease

[a] The first plant in each series was used to determine the natural infectivity of field-collected leafhoppers.

[b] Plant used as a source of virus.

ent diseased grapes and from 5 different diseased alfalfa plants to healthy grapes and alfalfa with 10 separate lots of naturally noninfective leafhoppers made up from one field collection of *Draeculacephala minerva*. In this series the virus of Pierce's disease was transmitted from diseased grapes to healthy

grapes in 5 out of 5 trials, and to healthy alfalfa in 4 out of 5 trials. The virus from dwarf-diseased alfalfa was also transmitted to healthy grapes and alfalfa the same number of times. Two alfalfa plants listed in table 5 died before readings were made. Similar experiments were performed with the other species of leafhoppers previously mentioned.

It is further evident from data shown in tables 4 and 5 that the incubation period of the virus in the leafhopper, if such exists, is less than 4 days.

Table 6 summarizes the transmission of the Pierce's disease virus from diseased to healthy grape, and from diseased to healthy alfalfa, together with intertransmissions between grape and alfalfa. The results reported in this table include transmissions with naturally viruliferous and naturally non-

TABLE 6.—*Transmission of the virus from diseased grape and alfalfa plants to healthy grape and alfalfa plants by four species of field-collected leafhoppers*[a]

Species of leafhopper	Transmissions from diseased grape						Transmissions from diseased alfalfa					
	To healthy grape			To healthy alfalfa			To healthy grape			To healthy alfalfa		
	No. of plants		Per cent infection	No. of plants		Per cent infection	No. of plants		Per cent infection	No. of plants		Per cent infection
	Inoculated	Infected		Inoculated	Infected		Inoculated	Infected		Inoculated	Infected	
Dracculacephala minerva	118	67	56.7	98	50	51.0	115	78	67.8	504	442	87.7
Carneocephala fulgida	69	41	59.4	95	40	42.1	42	30	71.4	112	69	61.6
Helochara delta	61	34	55.7	75	37	49.3	73	42	57.5	115	71	61.7
Neokolla circellata	159	122	76.7	97	54	55.6	73	53	72.6	62	35	56.4

[a] A composite of results of tests made from 1939 through 1943.

viruliferous leafhoppers, conducted from 1939 through 1943. In all these tests, wherever transmission was obtained the grape manifested Pierce's disease, and the alfalfa showed symptoms typical of dwarf. The data show that only one virus is associated with Pierce's disease of grape and dwarf of alfalfa.

In completing these transmission studies, many collections of the four species of leafhoppers were made in areas thought least apt to contain naturally viruliferous populations. To run natural infectivity tests with each small lot of leafhopper vectors used would have consumed large numbers of plants. Therefore, in order to obtain a measure of the numbers carrying the virus, natural infectivity tests were made with only random samples from each field collection (Table 7). According to table 7 and also tables 1 and 2, the 3 species of leafhoppers *Dracculacephala minerva, Helochara delta,* and

Carneocephala fulgida did to some extent carry the virus naturally under field conditions. When, however, a comparison is made with the high percentages of transmissions obtained after feeding these leafhoppers on diseased plants, one can reasonably assume that the possible numbers of naturally viruliferous leafhoppers used in the transmissions reported added but little to the error in the results.

With the species *Neokolla circellata*, 59 per cent of the lots tested proved to contain naturally infective leafhoppers. This high percentage adds con-

TABLE 7—*Results of tests with random samples from collections of four species of leafhoppers made to determine whether certain populations used in transmission trials were naturally infective*[a]

Species of leafhopper	Total number of leafhoppers in all collections	No. of lots tested	No. of lots naturally infective	Per cent of lots naturally infective
Draeculacephala minerva	6,650	38	5	13
Carneocephala fulgida	3,850	28	1	4
Helochara delta	2,000	13	1	8
Neokolla circellata	3,085	80	47	59

[a] Naturally infective means that the lot tested contained one or more viruliferous individuals when collected.

siderable error to the results obtained with transmissions from a known virus source with this species, except with lots previously proved not to be naturally infective.

SUMMARY AND CONCLUSIONS

The distribution and spread of alfalfa dwarf suggested transmission of the virus by insects.

Four species of leafhoppers—*Draeculacephala minerva* Ball, *Carneocephala fulgida* Nott., *Helochara delta* Oman, and *Neokolla circellata* (Baker)—were found that would transmit the alfalfa-dwarf virus from diseased to healthy plants.

The distribution of grapevines affected with Pierce's disease followed two general patterns: first, diseased vines irregularly scattered over the vineyard; second, diseased vines concentrated in small areas. Both types of distribution indicated that insects spread the virus.

The grouping of diseased vines under insect light traps added convincing evidence that the virus of Pierce's disease was transmitted by insects. Collections of insects made under the traps and compared with those made at some distance from the traps provided a valuable clue to the identity of the insects involved.

The same four species of leafhoppers that transmitted the alfalfa dwarf virus were at the same time proved capable of transmitting the virus of Pierce's disease.

Pierce's disease was usually more prevalent in vineyards of districts where considerable alfalfa was grown and in portions of vineyards adjacent

to alfalfa. Under such conditions the alfalfa fields were usually found infested with dwarf.

Root pieces from diseased grapevines inserted into the roots of alfalfa plants apparently transmitted the virus in 12 out of 35 trials, but root pieces from alfalfa similarly inserted in the stems of rooted grape cuttings did not transmit the virus to grapevines.

Naturally viruliferous field-collected leafhoppers of *Draeculacephala minerva, Carneocephala fulgida, Helochara delta,* and *Neokolla circellata* transmitted to healthy alfalfa and grape plants a virus producing, respectively, dwarf and Pierce's disease.

The incubation period of the virus in the leafhopper *D. minerva* is less than 4 days.

Naturally nonviruliferous field-collected leafhoppers of the same four species transmitted the virus of Pierce's disease from diseased grapes to healthy alfalfa and grape and from diseased alfalfa to healthy alfalfa and grape.

The majority of field collections of *Draeculacephala minerva, Carneocephala fulgida,* and *Helochara delta* were nonviruliferous, whereas 59 per cent of the lots of *Neokolla circellata* tested were naturally viruliferous.

According to the intertransmission experiments, the virus that causes Pierce's disease of grapes also causes dwarf of alfalfa.

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ELECTROPHORETIC STUDIES WITH THE PLANT VIRUSES[1]

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(Accepted for publication August 14, 1945)

Any scheme for the ultimate diagnosis of virus diseases in plants, or the identification of the viruses must, of necessity, involve the specific physical and chemical properties of the viruses themselves, in much the manner that the identification of bacteria involves their physiological behavior. Several means of testing for the presence of viruses in plants which have been suggested, other than that of testing the infectivity of the preparations, and which are based on the qualitative or quasi quantitative observations of the intensity of the action of oxidizing enzymes in plant extracts, on the determinations of quantities of ascorbic acid, glutathione, or plant pigments or similar products of plant metabolism in the plant sap, or on the accumulation and translocation of carbohydrates, or the nitrogen distribution in the plants, are of little value because these several tests are not specific for either the viruses or for the products of metabolism which are specific to any one of the virus diseases. In general, the color reactions with viruses and with proteins are so similar that differences in intensity of the reactions are extremely difficult to observe, and it is impossible by this means to distinguish one protein or virus from another. The picture relative to specific products of metabolism in the instances of the several viruses is obscure indeed, and attempts to correlate virus diseases with the diverse products of plant metabolism are entirely empirical.

There are, however, physical and chemical properties of the viruses and proteins—specifically, their electrokinetic properties—associated directly with the quality, quantity, and relative positions of the amino acids and other reactive units involved in virus and protein architecture, which may be made use of in diagnosis and identification. It is well known that colloidal particles will move through a fluid under the influence of an electric field. This migration is obtained by virtue of the electrostatic charges carried by the particles, and the charge in the surfaces of these particles is produced either through ionic adsorption or through ionization of the material making up the colloidal particle, or both. With proteins and with viruses the electrostatic charges in the surfaces of these entities are due to the ionization of the acidic and basic groups present, and the intensity of the net charge, resulting from the ionization, is related directly to the relative number of these active groups, as well as to the degree of their ionization, which in turn is a reflection of the architecture of the particles in question. The viruses and pro-

[1] This research has been terminated. The authors deeply regret that only an incomplete report may be made. Submitted December 17, 1944.

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teins are amphoteric, and the magnitude of the electrostatic charge carried by the particles or molecules is a function of the hydrogen ion activity in the solution in which they are dispersed; there is a specific pH-mobility behavior for each species of protein molecule or virus particle.

Given an aqueous protein solution placed in contact with the pure solvent in such a manner that there is a discontinuity in the physical properties at the interface, there will be generally, under electrophoresis, a migration of the region of discontinuity, or boundary, between the solution and solvent. The velocity of migration of the boundary will be characteristic of the protein in question, subject to the definition of conditions of pH, ionic concentration, temperature, viscosity of the solvent, and to the potential gradient. With a solution made up of two proteins there will be generally a boundary, or migration front, for each of the proteins. With a mixture of several proteins, there will be generally a boundary for each protein present in the mixture. These several migration fronts may be detected optically, and from such a study information may be obtained concerning the number of electrophoretically separable components in the mixture, the degree of electrical homogeneity of any component, and the concentration of any one of the components in the mixture. Where the components are separable, the pH-mobility behavior of each may be determined. It is also possible to isolate the individual components of such a mixture by electrophoresis.

The hypothesis which forms the basis of these studies is that with the extracts from plants infected with the viruses there will be specific migration fronts representative of the viruses involved, and that the viruses may be identified from their migration velocities under conditions defined with respect to pH, electrolyte concentration, and potential gradient.

METHODS

The electrophoretic experiments were carried out in a U-tube with a rectangular cross section, as designed by Tiselius (10) to effect maximum transfer to the bath of heat generated by the current passing through the U-tube. Analysis of the migration fronts was obtained by use of the adaptation by Lamm (4) and Toepler (11) of the device Foucault used as a means of accurately testing lenses for chromatic and spherical aberrations, together with the scanning camera designed by Longsworth (5).

The optical scheme, as illustrated in figure 1, for the analysis of the migration fronts is based on the refraction of light passing through the interface between the solution and the solvent. The U-tube (10) in which the determinations were carried out is arranged in sections so that the filling may be effected leaving a very sharp interface between the solvent and solution, and no difficulty is experienced in observing initially the discontinuity in the physical properties at the interface. The interface becomes diffuse in time, due in part to diffusion. L is the source of illumination. S is a horizontal slit about 1 mm. wide. The lens, D, which in the instance of the apparatus assembled at Cornell University was 4.5 inches in diameter and

had a focal length of 23 inches, forms the image of the slit S at the point P. E is one arm of the electrophoresis cell, and the region designated as A is the region between the solution *m* (plant extract in our case) and the buffer *b*. O is the camera objective, having a focal length of 23 inches,[4] and the camera is focused on the cell E. Under ordinary conditions all the light from the slit S, which is focused at the point P, enters the camera, and a clear image of the cell is formed on the photographic plate C. If there is a region in the cell where there is a continuous change in the index of refraction, as would be the case after the establishment of a concentration gradient, the light passing through that region will be refracted downward in a continuous manner through the width of the U-tube, because with each increment of distance traveled by the light beam through the region A, it enters a region of greater and greater refractive index. Consequently, the pencil of light passing through the region A will be focused at the point P′ rather than at P. If the refraction is not too great, the light focused at P′ will still enter the camera objective, and the image of the entire cell will be formed on the photographic plate. If, however, a knife edge Q is placed in a position im-

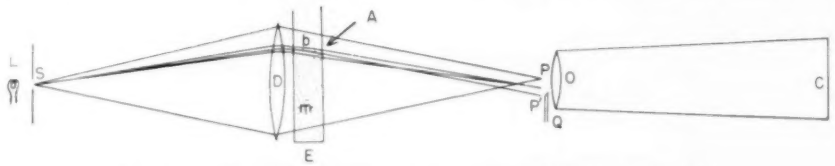

FIG. 1. Schematic diagram of the electrophoresis apparatus.

mediately below P, but above P′, the pencil of light focused at P′ will not enter the camera objective, and there will be an unexposed area on the photographic plate corresponding to the region A in the electrophoresis cell. This will be true for each region in the cell where there is a variation of the index of refraction with height, that is, for each migration front.

The variation of the index of refraction with height in the region A is not abrupt if there has been a lapse of time. The curve obtained by plotting the index of refraction against the height of the cell will be S-shaped in the region of A, and the rate of change of refractive index with height will be a maximum in the center of the S. The pencil of light passing through this particular region will be refracted most, as indicated in figure 1. If the knife edge Q is moved vertically, the shadow on the photographic plate will first be only a thin line, corresponding to the center of the region A, but the shadow will widen as the knife edge approaches P; when the knife edge has reached P, the shadow will correspond in width to the entire region A in the electrophoresis cell.

The rear of the camera is arranged as indicated in figure 2, where K is a vertical slit about 1 mm. wide. One arm of the tube is brought to a focus on one slit, the other being masked, and the exposed area of the plate corre-

[4] Approximate unit magnification of the cell is desired, and a long optical lever between the points E and O is desired in order to make the instrument sensitive to small changes in index of refraction in the cell.

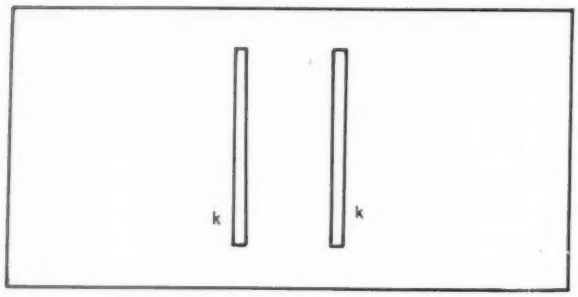

FIG. 2. Slits on the rear camera plate.

sponds to the area of the slit. Successive exposures of a plate at different positions along the rear of the camera, with the knife edge set progressively higher and higher with each exposure, results in a pattern of the type indicated in figure 3. Experimentally, the movement of the photographic plate is synchronized with the movement of the knife edge so that a continuous pattern is formed. These are the "scanning" patterns presented in this communication.

The concentration of each component in a mixture is proportional to the area of its shadow in the pattern on the photographic plate. It is also probable that there is a relation between the slenderness of the shadow, or peak, and the diffusion constant of the component.

Magnification of the image of the cell on the photographic plate was 1.3. The distance from the camera objective to the center of the cell was 42 inches, and from the center of the cell to the lens D was 11 inches. The photographic plate traveled 7.5 times faster than the knife edge Q.

The difficulty with electrophoresis of plant material has been in the preparation of the sample. Usually there is much pigment and suspended colloidal material in the extracts, which makes the photography difficult, and generally the concentrations of the components in the plant saps are too low for satisfactory scanning. It has been found useful in the preparation of the samples for electrophoresis to cytolyze the leaves with ether (1) initially. After cytolysis the vacuolar sap was pressed out with a hydraulic press and discarded. The vacuolar sap contains very little protein, and in tests with

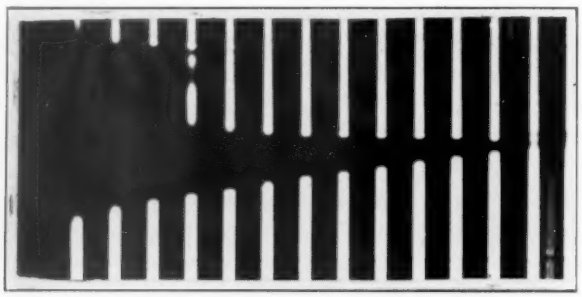

FIG. 3. Scanning pattern built up by successive exposures after alteration of the height of the knife edge and the position of the plate holder.

plants infected with tobacco mosaic virus, it was found to contain little or no virus. The discarded sap does carry much pigment, however, as well as substrate for oxidizing enzymes. The pressed material was then washed with water several times, and after each washing the excess water was pressed out with the press. These washings do not affect the character of the scanning patterns obtained with the healthy tobacco plant, but they do effect the removal of additional pigment. In some determinations the pressed and washed material was ground in a Waring blender in the presence of buffer. In these instances concentration of the extractable proteins was obtained by reusing the same buffer with successive portions of leaf material, so that about 100 cc. of buffer were used in extracting 600 grams of leaves. Further concentration was obtained by placing the extract in cellophane tubing and hanging it before a fan. It has been found, however, that more efficient extraction may be obtained by grinding the pressed leaf material in a food chopper and extracting it subsequently with the buffer. An observation which has been of significance in these studies is that the intensity of pigmentation in the extracts, and the amount of suspended material that will not sediment in the centrifuge, is related to the age of the plant. Best results are obtained with young plants. With reference to the extracts alluded to above, they were centrifuged at 3,600 r.p.m. to remove plant debris, and then dialyzed against 2 liters of 0.1 M phosphate buffer at 4° C. for a minimum of 18 hours. This same buffer was used as the solvent *b*, as shown in figure 1. After the dialysis was complete, the extracts were centrifuged at 16,000–25,000 r.p.m. for 3–5 minutes, in order to remove plastids and other bodies, before electrophoresis determinations were made.

The potato tubers were ground in the Waring blender in the presence of 0.10 M phosphate buffer at pH 7.5, together with a trace of KCN, and then the slurry was centrifuged at about 3,000 r.p.m. for about 15 minutes. The supernatant liquid was added to an equal volume of a saturated solution of ammonium sulphate, and was again centrifuged. This second supernatant liquid was discarded, the precipitate was taken up in 0.10 M phosphate buffer, and dialyzed against the same buffer for about 18 hours. The solution was then centrifuged at 16,000–25,000 r.p.m. for 3–5 minutes before the electrophoresis determinations were made.

These preparations were never free from pigment and suspended matter, and it was necessary in most cases to resort to infra-red photography.

EXPERIMENTAL RESULTS

The scanning patterns for both the ascending and descending[5] fronts in the U-tube for leaf extracts from healthy Turkish tobacco plants, at pH 7.5, are presented in figure 4, a and b. The time for electrophoresis was 30 and 60 minutes respectively. The indications are that there are only 3 protein

[5] The plant sap is in the bottom of the U-tube. Since the solute migrates in the electric field, one boundary moves up and the other moves down. These migration fronts are the ascending and descending migration fronts. The arrows in each figure indicate the direction of migration.

components extracted by the methods used, although it is probable that there are actually three groups of proteins, none of which has been resolved electrophoretically under the conditions used. The velocity of migration (all velocities reported are for the descending boundaries) of component A was 0.83×10^{-4} cm./sec./volt/cm., that of component B was 2.1×10^{-4} cm./sec./volt/cm., and that of component C was 5.2×10^{-4} cm./sec./volt/cm.

The patterns with the healthy tobacco plant are not affected with the age of the plant up to at least three months.

The patterns obtained with tobacco plants five days after inoculation with tobacco mosaic virus, and at a time when no symptoms were evident, were identical with those shown in figure 4, a and b. Nine days after inoculation vein clearing was evident in the leaves, and the patterns obtained with leaves harvested at this time are presented in figure 4, C. These data are for 30 minutes of electrophoresis, and the buffer used was identical with that used for the healthy plants. Components A, B, and C present in the extracts from the nine-day-diseased plants were identified by their migration velocities as being identical with the corresponding components in the extracts from the healthy plants. Component V, migrating with a velocity of 3.9×10^{-4} cm./sec./volt/cm., is new, and it is believed to be the virus.

Marked symptoms were evident in the tobacco plants fifteen days after inoculation. The patterns obtained with these severely diseased plants are presented in figure 4, d. Time for electrophoresis was 30 minutes, and the same buffer was used as in the determinations previously discussed. Components A and B were present in the extracts, as evidenced from the shadows in the scanning patterns, but component V had become overwhelmingly predominant. The method of extraction precludes precise determination of the concentrations of the several components in either healthy or diseased plants, but after dealing with many samples the authors believe that there is no substantial change in the concentrations of the components A, B, and C with the development of the disease in the tobacco plants. The patterns shown in figure 4, d, were obtained with much more dilute extracts than those in either a, b, or c of figure 4, and in more concentrated preparations, evidence has been obtained that component C is also present.

The evidence that the normal protein components in the extracts are not materially altered by the development of the disease is supported by the data obtained by serological methods (8), since antibodies engendered in experimental animals with sap from tobacco plants infected with tobacco mosaic virus are precipitated by sap from healthy tobacco plants.

The reasons for believing that component V is the virus are three-fold, namely, the component is present only in the infected plants and increases in quantity as the disease progresses, the area of the shadow on the photographic plate caused by this component is more than thirteen times the combined areas induced by the other components in the extracts (9), and the slenderness of the peaks is suggestive of a substance with a small diffusion constant (2, 3, 7).

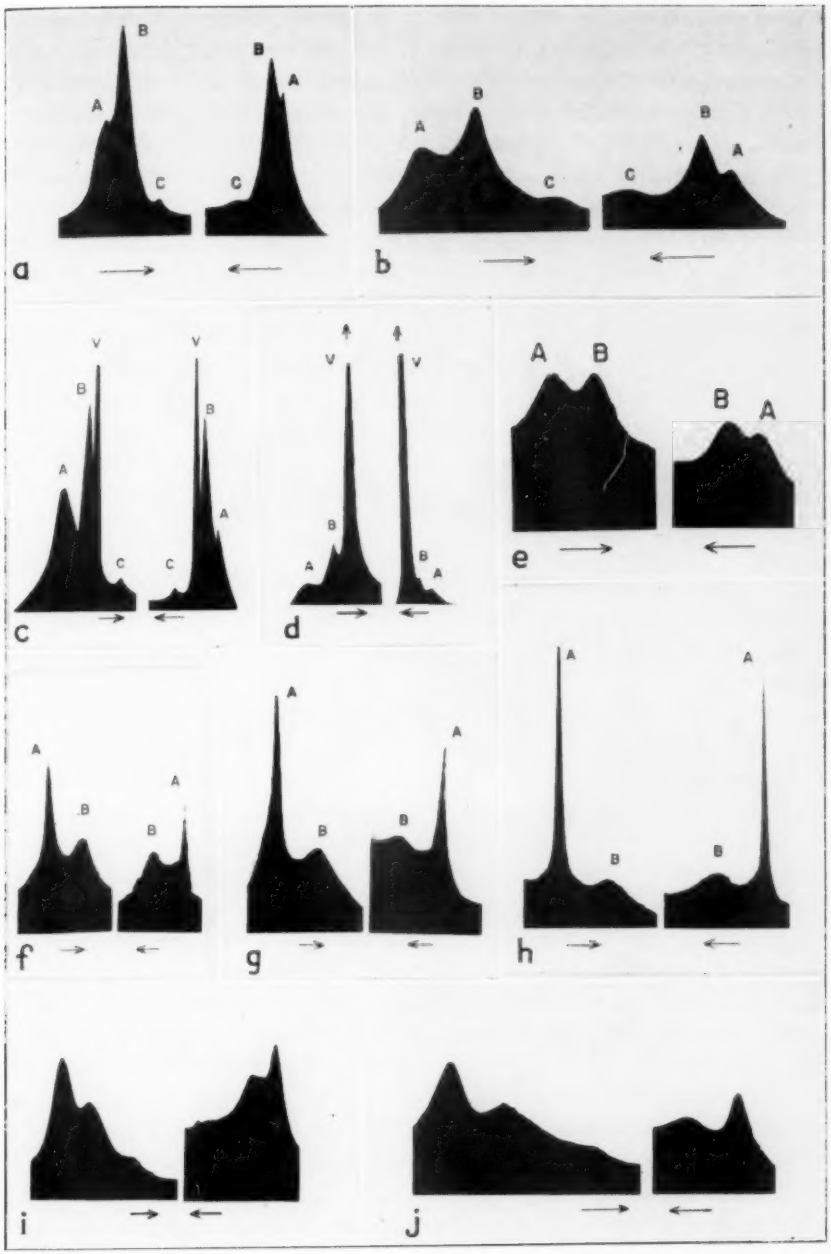

FIG. 4. Scanning patterns, descending at left, ascending at right. Healthy tobacco leaves with 30 min. electrophoresis (a) and with 60 min. electrophoresis (b). Tobacco leaves with tobacco mosaic virus nine days (c) and fifteen days (d) after inoculation. Healthy tobacco leaves (e) at pH 6.25. Tobacco leaves infected with the potato X virus at pH 7.5 (f), at pH 7.9 (g), and at pH 6.25 (h). Tobacco leaves infected with the potato Y virus, at pH 7.5, with 30 min. electrophoresis (i) and 60 min. electrophoresis (j).

The question may be raised regarding the completeness with which the virus may have invaded the plants after fifteen days, and the possibility may be suggested that the patterns shown in figure 4, d, were obtained with a mixture of diseased and healthy tissue. An attempt was made to answer this question. The severely diseased plants were cut back and two shoots from each were permitted to grow, as it was believed that in this manner leaf material might be developed under conditions where the opportunity for

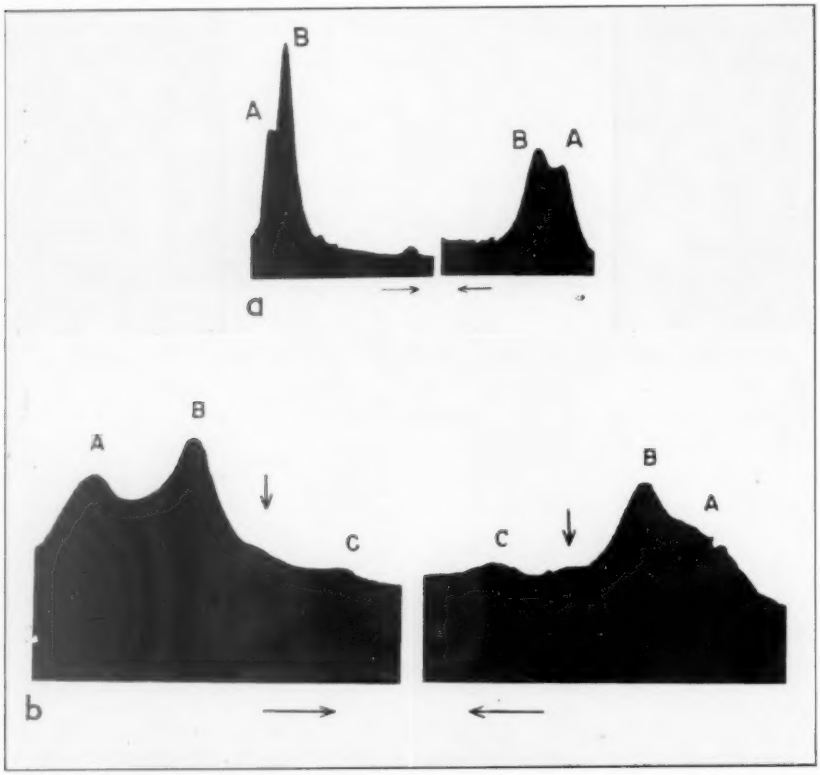

FIG. 5. Scanning patterns for tobacco leaves infected with Johnson's cucumber mosaic No. 1 (a) and with Price's indicator strain of cucumber mosaic No. 1 (b). Both are at pH 7.5.

systematic infection was maximum. These shoots were harvested after two weeks and the scanning patterns determined. They were identical with those presented in figure 4, d.

The abnormalities in the scanning patterns obtained with plants infected with other viruses are not so spectacular as those obtained with tobacco plants infected with tobacco mosaic virus. The patterns obtained with extracts from tobacco plants infected with potato X virus, which was obtained from potatoes, variety Green Mountain, are presented in figure 4, f. These data were obtained at pH 7.5. Time for electrophoresis was 60 minutes. In comparing these patterns with those obtained with the healthy plant, it

will be noted that the component A in these diseased plant extracts was present in a greater relative concentration than the corresponding component in the extract from the healthy plants. Its migration rate, at pH 7.5, was greater than that of the normal component A in figure 4, a and b. Component B, however, is the normal protein B in a and b of figure 4. The suspicion is that the normal component A and the X virus in the extracts are migrating with the same velocity under these conditions with respect to pH and electrolyte concentration. An attempt to separate, electrophoretically, the normal component A and the X virus by alteration of the pH of the buffer was without success. The patterns obtained at pH 7.9 are presented in figure 4, g, while those obtained at pH 6.25 are presented in figure 4, h. The patterns obtained with the healthy tobacco plant at pH 6.25 are presented in figure 4, e. A printable photograph for the patterns for the healthy plants at pH 7.9 is unavailable, but the patterns for the healthy plant as shown in a and b of figure 4 may serve as a control, since the +0.4 pH change caused but little alteration in the general shape of the pattern. The fate of component C had not been determined at the time the research was terminated.

The most reasonable interpretation of these data, with reference to component A, is that the virus has combined with the normal protein particles, imparting to them an altered electrophoretic mobility. This phenomenon is not uncommon with proteins (6).

The patterns obtained with extracts from tobacco plants infected with the potato Y mosaic are presented in figure 4, i and j. The time for electrophoresis was 30 and 60 minutes; the pH was 7.5. No opportunity was afforded for an attempt at separation of component A and the Y virus.

James Johnson's cucumber mosaic No. 1[6] was transferred to tobacco plants and after symptoms were well developed the plants were harvested and the scanning patterns (figure 5, a) were determined with the extracts from the diseased leaves. Phosphate buffer was used, at pH 7.5. Components A and B are the normal tobacco-leaf proteins. The rapidly moving component apparent in the ascending arm of the U-tube has not been identified, and the fate of component C was not ascertained.

Price's indicator strain of cucumber mosaic No. 1[6] was likewise transferred to tobacco plants, and the scanning patterns were determined with the diseased leaves, which were harvested after the symptoms in the plant were well developed. The data are presented in figure 5, b. Components A, B, and C are the normal tobacco-leaf proteins. The abnormal component at the head of the arrow migrated with a velocity of 4.0×10^{-4} cm./sec./volt/cm.

The patterns obtained with leaf extracts with the healthy pea bean, variety Otenashi, are presented in figure 6, a, whereas the patterns obtained with extracts from bean leaves infected with Zaumeyer's bean virus No. 4[7] are presented in figure 6, b. Components A and B in the two scanning patterns migrated with velocities of -0.25×10^{-4} cm./sec./volt/cm. and $+1.9 \times 10^{-4}$

[6] Kindly given to us by Dr. H. M. Munger.
[7] Kindly given to us by Dr. B. L. Richards, Jr.

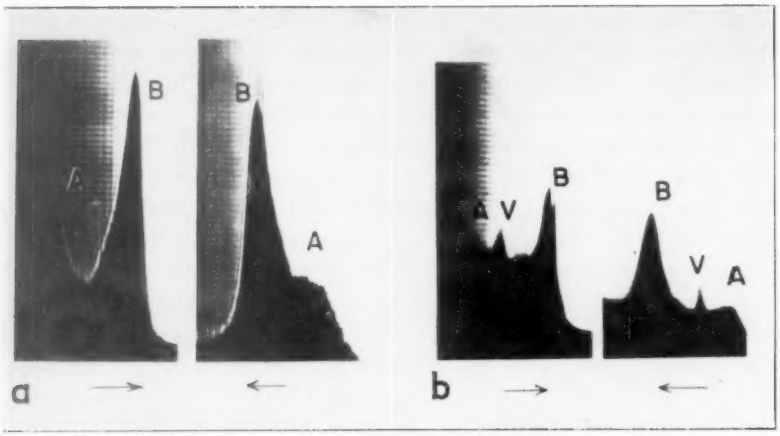

FIG. 6. Scanning patterns for healthy pea bean (a) and for pea bean leaves infected with Zaumeyer's bean virus No. 4 (b). Both are at pH 7.5.

cm./sec./volt/cm. respectively, and component V in figure 6, a, migrated with a velocity of 0.90×10^{-4} cm./sec./volt/cm.

The patterns obtained at pH 7.5 with virus-free potato tubers are presented in figure 7, a. These patterns are not influenced by the time of storage of the tubers, and they are the same for all the virus-free tubers we worked with. Field-grown tubers present complications which have not been resolved, since most viruses with which they may become infected will remain in the stock, and these tubers, although they may appear healthy, frequently carry more than one virus. Patterns obtained with Green Mountain and Cobbler varieties of potatoes which were reputed to be healthy are presented in figure 7, b and c, respectively. The opportunity has not been available for the characterization of the two additional components which are obviously present in the extracts, but it is suspected that one of them is the X mosaic.

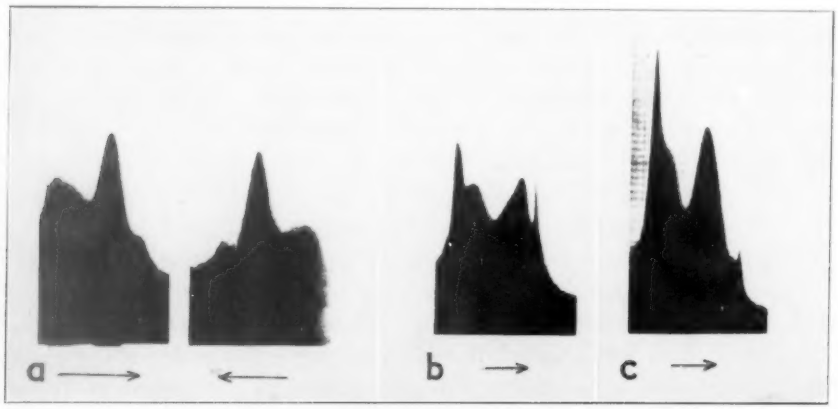

FIG. 7. Scanning patterns for healthy potato tubers (a), for field-grown tubers of Green Mountain potato (b), and for field-grown tubers of Cobbler potato (c). All are at pH 7.5.

DISCUSSION

Considered from the point of view of control of plant viruses, the initial concern should be, of course, with a general scheme for the diagnosis of virus diseases and the positive identification of the viruses. Ultimately, however, the control will be obtained through breeding programs based on a thorough understanding of the nature of resistance of plants to virus diseases and of the mechanism of immunity. There can be no question but that the key to that understanding will be in the dynamics of the leaf proteins, and it will be found through the employment of chemical science; the chemistry of the leaf proteins and plant protein metabolism are fundamental to the problem, and one cannot expect substantial progress in the control of these diseases by employing the empirical methods of the "practical" pathologist.

Our investigations had not reached the point where mixtures of viruses were involved when the investigation was terminated, but with each of the readily sap-transmissible viruses worked with, the patterns obtained are distinctive. In no case can it be said that the abnormal components in the several plant saps have been established as the respective virus components concerned, but the presumptive evidence that such is the case is strong. There is every reason to believe that the scheme used herewith may be used generally in diagnosis of virus diseases in plants and for the positive identification of viruses in infected plants. There are, however, technical difficulties which must be resolved.

The methods available for the extraction of leaf proteins are far from satisfactory, and the total quantity of protein extracted in any case is only a fraction of the total leaf protein. Those normal leaf proteins which have been extracted are difficult to handle; none of them have ever been crystallized as they do not lend themselves readily to manipulation. Concerning the methods of extraction, the use of ether for cytolysis appears to yield the best results for extraction in general, but, although ether appears to have no effect on the extractable proteins from the healthy tobacco plant, little is known regarding its effects on other plant proteins, nor is it known what influence it may have on the viruses. As has been indicated, the great virtue with the use of ether is in the elimination of much pigment, and, with the elimination of the vacuolar sap, a substantial concentration of the extractable proteins is obtained. The extraction of leaf proteins with solutions of electrolytes, with the exception of the dilute buffer solutions, has been neglected, and the same is true with respect to dilute acids and bases. The use of the Waring blender has no influence on the patterns obtained with the healthy tobacco plant, tobacco plants infected with tobacco mosaic virus, or with the virus-free potato tubers, but the possible influence of the blender on the surface denaturation of extractable proteins from other plants has not been ascertained.

These remarks regarding problems involved with reference to the leaf proteins apply to the viruses. Virtually nothing is known regarding the

physical and chemical properties of the viruses. With the exception of the few which have been isolated, very little may be said concerning the conditions under which they become soluble; their tendencies to become adsorbed on inert surfaces, such as cell debris produced during extraction; the ease with which they become denatured; their chemical reactivities or composition; their relative concentrations in the plant saps; and nothing is known regarding the rôle they may play in the production of disease.

The efforts to obtain scanning patterns have been limited in this study to a few of the viruses which are readily sap-transmissible. No attempt has been made to work with viruses which are not readily sap-transmissible or with the viriferous or nonviriferous insects known to be vectors of the viruses. No opportunity has been available for consideration of seeds infected with seed transmissible viruses.

Two suggestions are offered in conclusion. In the first place the pH-mobility curves of the viruses should be determined in so far as it is possible to do so—and many of these data may be obtained without the isolation of the viruses in question—as these data will be of considerable value in any sound scheme for the classification of the viruses, since the mobility behaviors are related specifically to their chemical composition and structure. The second suggestion comes from the observation that the patterns produced by plants belonging to the same genus have remarkably similar patterns. It would be worth while to determine the degree of similarity in the several genera.

SUMMARY

1. The moving boundary method of electrophoresis has been outlined.

2. Specific scanning patterns have been obtained for the extracts of healthy tobacco plants and for tobacco plants infected with tobacco-mosaic virus, with the potato X and potato Y viruses, with James Johnson's cucumber mosaic No. 1, and with Price's indicator strain of cucumber mosaic No. 1. These patterns are not identical.

3. Specific scanning patterns have been obtained for healthy pea beans, variety Otenashi, and for the pea bean infected with Zaumeyer's bean virus No. 4.

4. Extracts from healthy tobacco plants contain three proteins, and their concentrations and nature are not affected by the development of tobacco mosaic in the plant. The appearance of the abnormality in the scanning pattern obtained with tobacco plants infected with tobacco mosaic virus is correlated in time with the appearance of symptoms in the plant.

5. Two proteins are found in the extracts of the healthy pea bean.

6. The scanning patterns obtained with potato tubers (virus-free from virus-free seedlings) are the same from seedling to seedling, and are not influenced by the time of storage. Patterns from apparently healthy field-grown tubers have been observed to have more than one abnormality.

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TWO LEGUME VIRUSES TRANSMISSIBLE TO TOBACCO[1]

E. M. JOHNSON

(Accepted for publication August 20, 1945)

Several virus diseases of alfalfa, *Medicago sativa* L., and white clover, *Trifolium repens* L., have been described and named.[2, 3, 4, 5] Most of these have a rather wide host range among the legumes. Virus diseases of both plants occur in Kentucky, some of which may be similar to those described. Inoculation and physical-property studies of a virus disease of alfalfa and one of white clover, occurring in Kentucky, are described in this paper.

ALFALFA VIRUS

An occasional alfalfa plant with inconspicuous virus patterns has been seen in a few fields in Kentucky. Affected plants have a few irregular, often indistinct chlorotic blotches on an occasional otherwise normal leaflet (Fig. 1, B). The virus is mechanically transmissible to tobacco and several other species, with or without the aid of carborundum, if inoculations are made in the greenhouse to actively growing young plants during late fall or early spring but not during the summer or winter, possibly because of the effect of a higher temperature on virus content. The symptoms on various plants are described in table 1, and some are illustrated in figure 1, A, B, and D and figure 3, A. Physical properties of the virus are given in table 2.

The alfalfa virus is transmissible from alfalfa to alfalfa by aphids but, in these tests, not by mechanical methods even when carborundum was used. Aphids were collected on alfalfa in the field and placed on an affected alfalfa plant in the greenhouse. A few days later these were removed to 10 disease-free alfalfa seedlings. After several weeks all the alfalfa plants showed symptoms typical of the alfalfa mosaic. When inoculations were made to tobacco with this material, typical symptoms developed. Twelve healthy alfalfa seedlings were set out-of-doors in an isolated area on April 2. At the same time an alfalfa plant affected with alfalfa virus was set at each end of the row. On April 10, aphids were numerous on all plants. In November all the alfalfa plants showed typical symptoms of the alfalfa mosaic. Inoculations, made the following spring from these plants to tobacco, resulted in typical symptoms of alfalfa mosaic.

June Pink and Marglobe varieties of tomatoes, when inoculated with alfalfa virus, develop only small necrotic spots on rubbed leaflets. When, however, tomato cions are grafted on alfalfa-virus-affected tobacco, the cions

[1] The investigation reported in this paper is in connection with a project of The Kentucky Agricultural Experiment Station and is published by permission of the Director.

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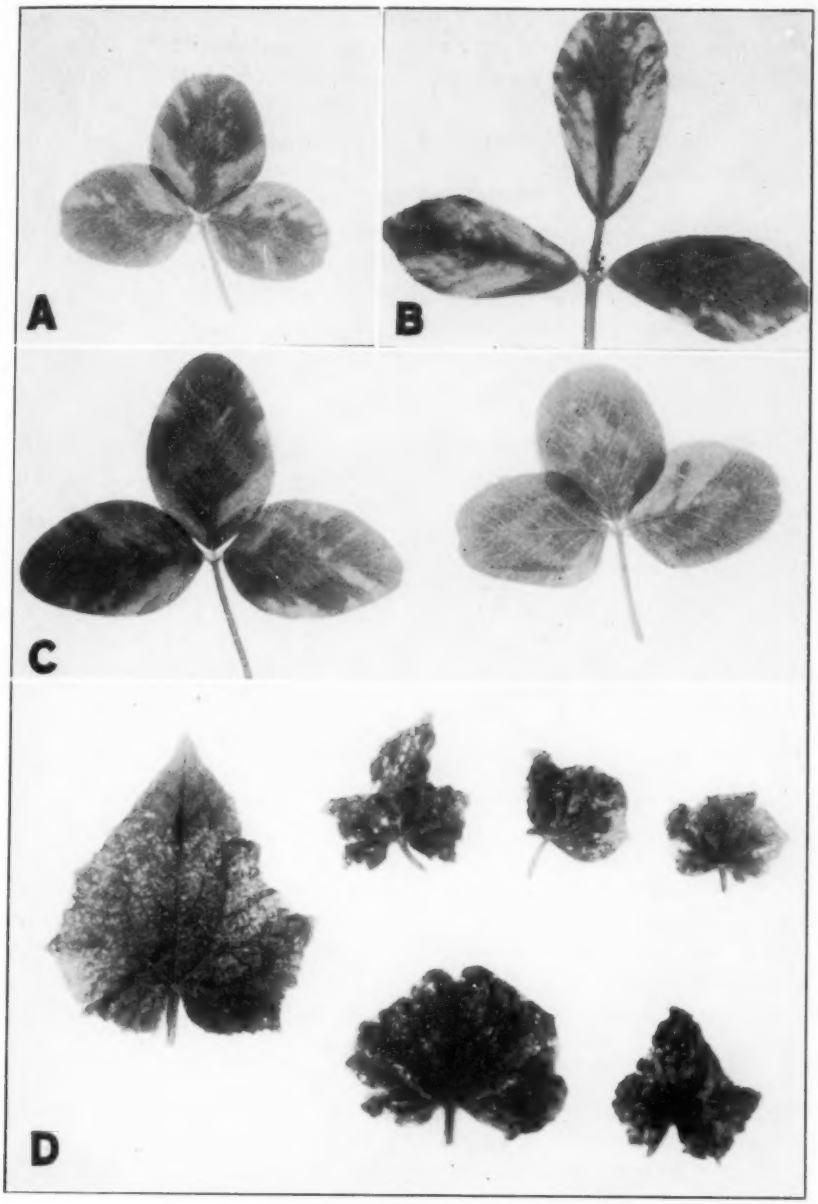

FIG. 1. A. Red-clover leaf affected with alfalfa virus. B. Alfalfa leaf showing typical mottling caused by alfalfa virus. C. Red-clover leaves affected with white-clover virus. D. Cucumber leaves showing mottling and distortion caused by alfalfa virus. All leaves from same plant.

show striking symptoms. Necrotic streaks develop on stems and petioles, the leaflets are small and crinkled and their margins are rolled downward. The leaves curve toward the stem giving the entire cion a dwarfed, bunched appearance.

Dodder (*Cuscuta sp.*), well established on a tobacco plant affected with alfalfa virus, was trained to a virus-free cucumber seedling. Four weeks later the cucumber plant developed symptoms typical of the virus (Fig. 1, D). Inoculations from this cucumber plant to tobacco resulted in symptoms typical of those produced by the alfalfa virus.

WHITE-CLOVER VIRUS

A virus disease of white clover has been seen for several years in small areas in an occasional pasture or lawn. Affected plants have irregular, pale yellow, mottled areas between the veins on a few to several, otherwise normal leaves. The virus is mechanically transmissible to tobacco and a few other plants (Table 1) if inoculations are made in late fall or early spring (Fig. 1, C, and Fig. 3, B).

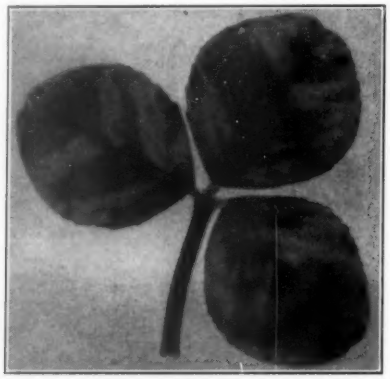

FIG. 2. White-clover leaf affected with the white-clover virus. Natural infection.

Attempts to infect white-clover plants mechanically with the virus from affected white clover, red clover, tobacco, garden peas, and garden beans have been unsuccessful. The virus is transmissible to red clover from any of the species mechanically infected with the virus (Fig. 1, C). No studies were made on dodder or aphid transmission of this virus.

COMPARISON OF ALFALFA VIRUS AND WHITE-CLOVER VIRUS

Although, in these studies, the alfalfa virus appears to have a wider host range than the white-clover virus the symptoms of both appear very much alike on plants susceptible to both viruses. This resemblance is sometimes striking on tobacco plants that have been infected for some time. On red clover and garden peas the symptoms are identical. The diseases resemble each other in that symptoms are masked at high temperatures. Neither virus can be transmitted mechanically, even with the aid of carborundum, when the temperature is about 80° F. or above. The physical properties are very much alike (Table 2).

Photographs and descriptions of the alfalfa virus on certain hosts were sent to Dr. W. J. Zaumeyer, who states: "The mottling of the alfalfa leaves

looks typical of the symptoms produced by the common alfalfa mosaic virus, but that means little since I feel that there are many strains of this virus.

TABLE 1.—*Means of transmission of alfalfa and white-clover viruses and the symptoms produced in various plants*

Host plant	Alfalfa virus		White-clover virus	
	Means of transmission	Symptoms	Means of transmission	Symptoms
Alfalfa, Grimm	Aphids, not mechanical	Systemic mottling	Not mechanical	.....
Red clover, Ky. 101	Rubbing	do	Rubbing	Systemic mottling
White clover	do	do	Not mechanical	Systemic mottling
Pea, Dwarf Telephone	do	Systemic mottling, Stem and petiole necrotic streaks, Dwarfing, Distortion	Rubbing	Systemic mottling, Stem and petiole necrotic streaks, Dwarfing, Distortion
Bean, Stringless Green Refugee	do	Necrotic spots on rubbed leaves	do	Systemic mottling, Necrotic spots on rubbed leaves
Tobacco, Ky. 16	do	Systemic mottling, Necrotic or chlorotic ring and line patterns, Dwarfing, Distortion	do	Systemic mottling, Dwarfing, Distortion, Necrotic or chlorotic line patterns
Tomato, June Pink and Marglobe	Rubbing, Grafting	Necrotic spots on rubbed leaves, Stem and petiole necrotic streaks, Dwarfing, Distortion	Not mechanical	.....
Zinnia, Pompon	Rubbing	Chlorotic or necrotic spots on rubbed leaves, Dwarfing, Distortion	do	.....
Cucumber, Long Green	Rubbing; Dodder	Systemic mottling, Chlorotic or necrotic spots on rubbed leaves, Dwarfing, Distortion	do	.....
Pokeweed, *Phytolacca americana*	Rubbing	Necrotic spots on rubbed leaves	do	.....
Pepper, California Wonder	do	Systemic mottling, Necrotic or chlorotic ring and line patterns, Dwarfing, Distortion	do	.....

. . . The virus which you have is apparently a strain of alfalfa mosaic and different from those which I have dealt with. The ones which I published

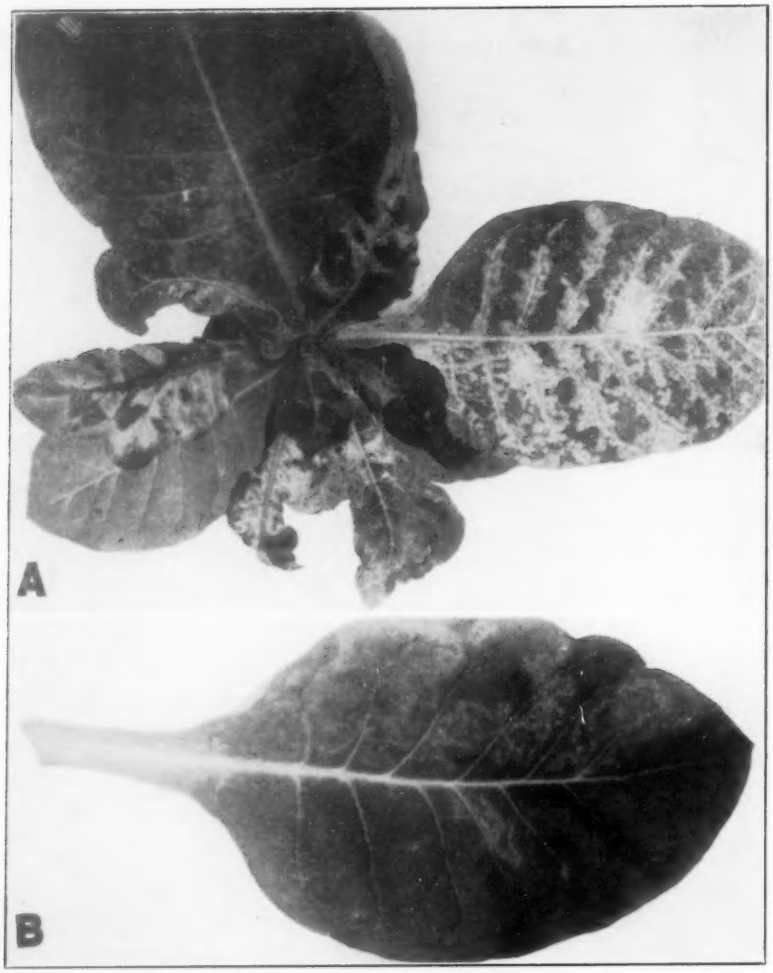

FIG. 3. A. Burley tobacco affected with alfalfa virus. B. Leaf of Burley tobacco affected with white-clover virus.

TABLE 2.—*Physical properties of alfalfa and white-clover viruses*

Physical properties	Alfalfa-virus infection		White-clover-virus infection	
	Positive	Negative	Positive	Negative
Tolerance to dilution	1: 100	1: 500	1: 100	1: 500
Thermal inactivation	58° C. (10 min.)	60° C. (10 min.)	55° C. (10 min.)	58° C. (10 min.)
Resistance to aging in vitro at room temperature	72 hrs.	96 hrs.	96 hrs.	120 hrs.
Resistance to drying at room temperature	15 days	30 days	15 days	30 days

on were not infectious to tomato, however, they did infect tobacco and cucumber but the symptoms on the latter were not as severe as yours."

The white-clover virus does not appear to be the same as F. Johnson's white-clover mosaic. Both viruses of his complex, pea mottle and pea wilt, have greater dilution values than the white-clover virus herein described and they affect neither tobacco nor tomato. From the brief studies reported in this paper, the alfalfa and white-clover viruses, tentatively, may be considered strains of alfalfa mosaic and related to the alfalfa mosaic virus described by Zaumeyer and Wade.

SUMMARY

A virus disease of alfalfa and one of white clover occurring in fields are described. Although the two viruses have different host ranges they resemble each other in symptomology on tobacco, peas, and red clover. The physical properties appear very similar. Both appear to be strains of one virus, probably the alfalfa mosaic.

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CANKER OF HYBRID POPLAR CLONES IN THE UNITED STATES, CAUSED BY SEPTORIA MUSIVA

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(Accepted for publication September 1, 1945)

In the late summer of 1941, specimens of a branch and stem canker (Fig. 1, A) on clones of hybrid poplars, collected at a plantation of the Soil Conservation Service, Attica, New York, and at the New York State Nursery, Saratoga Springs, New York,[2] were referred to the writer for examination and a determination of the causal organism. The disease was reported to be prevalent in these experimental plantings on many of the clones of hybrid poplars that have been developed by Schreiner and Stout (10, 11) for pulpwood reforestation. The cankers did not resemble those characteristic of the common poplar diseases caused by *Dothichiza populea* Sacc. and Briard and *Cytospora chrysosperma* Fr., although mature pycnidia of *Cytospora* were present on or surrounding some of the cankered areas. Sporodochia of a species of *Fusarium* were conspicuous on a few of the cankers, but, in a superficial examination, no fungus could be found consistently in the diseased areas. On two of the cankers from the Attica collection a few small black pycnidia containing hyaline, curved, septate spores suggestive of a *Septoria* were present. The same fungus was obtained also in tissue cultures from a number of cankers in both collections. The *Septoria* corresponded with the description of *S. musiva* Pk. (8) that Bier (1) has recently reported as the cause of a serious canker disease of hybrid poplars in Canada. Cankers similar to those on the Attica specimens were recently collected[3] from hybrid poplar clones planted by the Tennessee Valley Authority at Norris, Tennessee. No fruiting bodies of *S. musiva* were observed on the cankers, but tissue cultures developed typical pycnidia and spores.

Because of the apparent severity and importance of the disease on clones of hybrid poplars that have proved particularly adaptable for reforestation, the present paper gives a brief review of the hosts, the distribution of the disease, the symptoms, the causal fungus, and the results of inoculations to test the relative susceptibility of a few of the clones.

HOSTS AND DISTRIBUTION

A canker disease of Russian poplars (probably *Populus petrowskyana* Schneid.) grown in experimental plantings at the Northern Great Plains

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[2] These collections were received respectively from E. J. Schreiner, Senior Silviculturist, Northeastern Forest Experiment Station, Forest Service, U. S. Department of Agriculture, Philadelphia, Pa., and from J. R. Hansbrough, Pathologist, Division of Forest Pathology, Bureau of Plant Industry, Soils and Agricultural Engineering, Agricultural Research Administration, U. S. Department of Agriculture, New Haven, Conn.

[3] The collection was made by G. F. Gravatt, Senior Pathologist, and the cultures were referred to the writer by R. W. Davidson, Pathologist, both of the Division of Forest Pathology, Bureau of Plant Industry, Soils and Agricultural Engineering, Agricultural Research Administration, U. S. Department of Agriculture, Beltsville, Maryland.

Field Station, Mandan, North Dakota, was reported by Johnson and Cobb (6) in 1923. The cause of the disease was unknown but it was described as "a disease which soon girdles the tree, generally near the base or at a crotch, and kills the portion above the canker." In 1936 the Northwest poplar, a rapid-growing hybrid, presumably balsam poplar (*P. tacamahaca* Mill.) × cottonwood (probably *P. deltoides* Marsh.), and considered to be native in North Dakota, was described by George (5) as "subject to canker." In Canada, the importance of canker on poplars in Saskatchewan has been emphasized (3, p. 28) and a brief study of the disease and its cause was made at Indian Head, Saskatchewan (4, pp. 24–26). An unidentified species of *Septoria* was isolated from leaf spots and cankers, and the susceptibility of *P. petrowskyana* and Northwest poplar was proved by wound inoculations of cuttings in the greenhouse.

A more detailed study of the disease at Indian Head and at the Petawawa Forest Experiment Station, Ontario, Canada, was reported by Bier in 1939 (1). He identified the causal fungus as *Septoria musiva,* which was described by Peck (8) in 1882 on living leaves of *Populus deltoides* (*P. monilifera* Ait.) at Albany, N. Y., and which is indigenous in North America. Bier's inoculations of cuttings in the greenhouse proved the susceptibility of the hybrid poplars *Populus rasumowskyana* Schneid., *P. petrowskyana,* and *P. berolinensis* Dipp. to stem infection. The Northwest poplar and another hybrid known as Saskatchewan poplar, with a similar parentage, also were susceptible. However, the native poplars *P. tacamahaca* and *P. deltoides* (designated by Bier as *P. balsamifera* L.), from which these hybrids were derived, proved to be resistant to canker formation. He found that leaf lesions were universally present on plantation trees susceptible to canker, and proved, by inoculations in the greenhouse, the susceptibility of the two species of native poplars, as well as the hybrid poplars, to leaf infection.

Thompson (12) studied *Septoria musiva* as the cause of leaf spot of poplars in the United States, and reported a twig lesion only on one shoot of *Populus szechuanica* Schneid. From his inoculations of poplar cuttings in the greenhouse, he found that 26 species and varieties are susceptible to leaf infection, including *P. maximowiczii* Henry and the Russian hybrids *P. petrowskyana* and *P. berolinensis.*

Recently a canker disease of poplars in Argentina was described by Sarasola (9) as caused by *Septoria musiva* and observed for the first time in that country in 1941. The origin of the fungus in South America is not yet known. Several hybrids and varieties of poplars, including clones developed by Schreiner and Stout (11), were reported as susceptible. The species *Populus laurifolia* Ledeb. and *P. simonii* Carr. (*P. przewalskii* Maxim.) also were reported (9) as susceptible. Leaf spots from which the same fungus was isolated occurred on the susceptible trees.

SYMPTOMS AND CAUSAL FUNGUS

The relation of leaf infection to the development of cankers on the hybrid poplar clones in the New York plantations has not yet been determined.

Bier (1) stated that spring infection of leaves and twigs of the current year's growth is brought about by ascospores of the perfect stage (*Mycosphaerella populorum* G. E. Thompson) produced in fallen leaves or in dead leaves that have remained over winter on affected twigs. This indicates the importance of leaf infection in the spread of the canker disease. Bier also found that, in the spring, mature perithecia and pycnidia with viable spores are sometimes present on cankers formed the previous year. Infection of newly developed leaves is evident by the presence of small, brown, circular or angular spots with yellowish or white centers. Several spots may eventually coalesce and increase in size until large areas are affected. Small black pycnidia appear in the lesions on both leaf surfaces. The leaves on the lower branches are most commonly affected, and early defoliation of these branches may occur. Infection of the twigs, as indicated by the specimens from the New York plantations, may take place through lenticels or wounds on the current season's growth. Bier (1) also reports infection through petioles and stipules. The bark of young lesions is dark brown or black and depressed, with a whitish central area in which small black pycnidia soon appear. On the most susceptible poplars the rapid spread of the fungus results in the girdling and death of the twig during the late summer. The fungus continues to grow from the axillary twigs into the main stem, on which perennial cankers may be formed. The presence of cankers around several leaf axils along the stem, with the swelling of the stem at the margins of the cankers and the constriction in the affected areas, results in a pronounced distortion of the stem. Pycnidia of *Septoria musiva* are rarely found on these cankers, but the dead bark and marginal callus tissue may be invaded by secondary fungi, particularly a species of *Cytospora*, probably *C. chrysosperma*. The continued development of the cankers eventually results in girdling and death of the tree. In the more resistant trees, the cankers develop more slowly and in some cases eventually become overgrown with callus tissue, with no further spread or girdling of the stem.

Bier (1) and Thompson (12) give brief discussions of the taxonomy and morphology of *S. musiva*, and the characteristics of the fungus in the present study, on the hosts and in culture, agree with their descriptions. The spore measurements for conidia are as follows: From pycnidia on leaves—Bier's measurements (330 spores from 4 hosts) 17.2–51.6 × 3–4 μ, Thompson's measurements (850 spores from 7 hosts) 28–54 × 3.5–4 μ. From pycnidia on cankers—Bier's measurements (180 spores from 3 hosts) 21.5–55.6 × 3–4 μ, the present study (200 spores from 2 clones) 24–56 × 3.5–4 μ. Sarasola (9) gives measurements of 1200 spores from pycnidia on the leaves of various hosts as 18.5–70 × 2.0–4.9 μ. This latter range is indicative of the wide variation in spore length mentioned by Davis (2), who considered *S. musiva* as a composite species. Thompson (12) described *S. populicola* Pk. as distinct from *S. musiva*, having a range in spore length of 45–80 μ, with an average of 62 μ. The average of spore length reported by Sarasola was 43.4 μ, which would seem to place his fungus in *S. musiva*.

In the present study the causal fungus was isolated and grown on Leonian's medium (7), from single spores produced in pycnidia on the cankers in the Attica collection and from the inner bark of nonfruiting cankers of both collections. The colonies grew slowly and produced pycnidia and spores during a comparatively short period, about 2 or 3 months. The entire surface of the colony then became overgrown with a profuse white or pinkish mycelium and no further fruiting took place. This mycelium grew readily in transfers, without the production of pycnidia or spores. Transfers by means of single spores from fruiting cultures produced mature pycnidia for some time, but eventually, after about 8 or 10 months, transfers of this type also failed to sporulate. Repeated attempts to obtain fruiting pycnidia by transfers of the mycelium to sterilized poplar twigs failed. However, when the mycelium from the Attica isolations was inoculated into living twigs of susceptible trees, cankers and typical fruiting bodies were formed. The fruiting period of the isolations from the Saratoga specimens was shorter than that from the Attica specimens and in inoculations the mycelium failed to produce fruiting bodies on the cankers. Bier (1) and Thompson (12) found that in culture some colonies never form pycnidia and spores.

INOCULATIONS

Three series of inoculations were made on cuttings from 10 clones of hybrid poplars selected by Schreiner for their vigorous growth and for their apparent variation in susceptibility to the disease in the nursery. The parentage of the clones is as follows:

OP–1	*Populus nigra* L.	×	*P. laurifolia* Ledeb.
OP–2	do	×	do
OP–4	do	×	do
OP–11	do	×	*P. trichocarpa* Hook.
OP–12	do	×	var. *betulifolia* (Pursh) Torr. × *P. trichocarpa*
OP–41	*P. maximowiczii* Henry	×	*P. trichocarpa*
OP–46	do	×	*P. berolinensis* Dipp.
OP–47	do	×	do
OP–52	do	×	*P. nigra* var. *plantierensis* (Simon-Louis) Schneid.
OP–55	*P. candicans* Ait.	×	*P. berolinensis*

The cuttings were rooted and potted in the greenhouse, and the current season's growth was inoculated in June. The inoculum consisted of pieces of sporulating pycnidia from the single-spore cultures from the Attica specimens and from tissue cultures from the Saratoga specimens. Ten inoculations (1 on each clone) were made by placing the inoculum from the Attica isolate in uninjured leaf axils. In 10 additional inoculations with the Attica isolate the inoculum was placed in contact with the exposed wood of small triangular stem wounds made with a sterile scalpel. Two similar series of inoculations were made with inoculum from the Saratoga isolate. The inoculated areas in all cases were protected by wrappings of wet cotton and waxed paper tied at both ends, the wrappings being removed after 10 days. A control, consisting of a stem wound without the insertion of the inoculum, was made for each pair of wound inoculations.

From the wound inoculations with inoculum from the Attica isolate positive results were obtained on clones OP–2, 46, 47, and 52. Typical cankers and mature pycnidia were formed, and *Septoria musiva* was reisolated in single-spore cultures in September. On clones OP–4, 11, 12, and 41 open wounds resulted, but no fruiting bodies of *S. musiva* were present and the fungus failed to develop in tissue cultures from the wounds. This may have been due to the fact that other fungi, such as *Fusarium* and *Cytospora*, were abundant and may have overgrown the more slowly developing *Septoria*. The wounds on OP–1 and 55 healed normally, as did those on all the controls. On clone OP–4 infection occurred through a leaf petiole from the inoculum placed in the leaf axil, and *S. musiva* was reisolated from spores in pycnidia on a small stem canker.

The inoculations with the Saratoga isolate produced partially healed wounds on OP–2, 11, 41, 46, 47, 52, and 55, with no evidence of pycnidia of *Septoria musiva* and the fungus failed to develop in tissue cultures from the wounds. On clones OP–1 and 12 the wounds inoculated with this isolate healed over in the same manner as the controls. Because of the failure to obtain positive results with this isolate it was not used in the two following series of inoculations.

The uninoculated cuttings remained in an unheated greenhouse during the winter and were planted outdoors the following spring. Two series of inoculations were made in June, one, on the stems of the previous year's growth, consisting of the application of mycelium to the exposed wood of stem wounds as in the preceding experiment, and the other, on the current season's growth, of a spore suspension on uninjured leaves and petioles, and on stem wounds. The wound inoculations were protected with wet cotton and waxed paper wrappings for 10 days in the first series and for 72 hours in the second series. The inoculum was derived from single-spore cultures from the Attica isolate. In the first series 2 stem inoculations were made on each of the 10 clones. In both series, controls were made on each clone to correspond with the different types of inoculations.

The cankers resulting from the inoculations in the first outdoor series were collected in September for examination and reisolation of the fungus. *Septoria musiva* was reisolated in single-spore cultures from the two cankers on OP–2 and from one canker on OP–46, 47, and 52 (Fig. 1, B, C). Fruiting bodies of a species of *Fusarium* were present on the second canker on each of these three latter clones, and no other fungus was obtained from tissue cultures. The two cankers on OP–12 and 41, and one on OP–4 were overgrown with a species of *Cytospora* and of *Phomopsis*. The second canker on OP–4 and the two cankers on OP–11 and 55 had healed.

The cankers in the second outdoor series, resulting from infection of stem wounds, were collected and the fungus reisolated one month after inoculation. At this time reisolations were made also from the leaf spots and petiole infections. In this series no secondary fungi had invaded the inoculated stem wounds and mature pycnidia of *Septoria musiva* were present, except

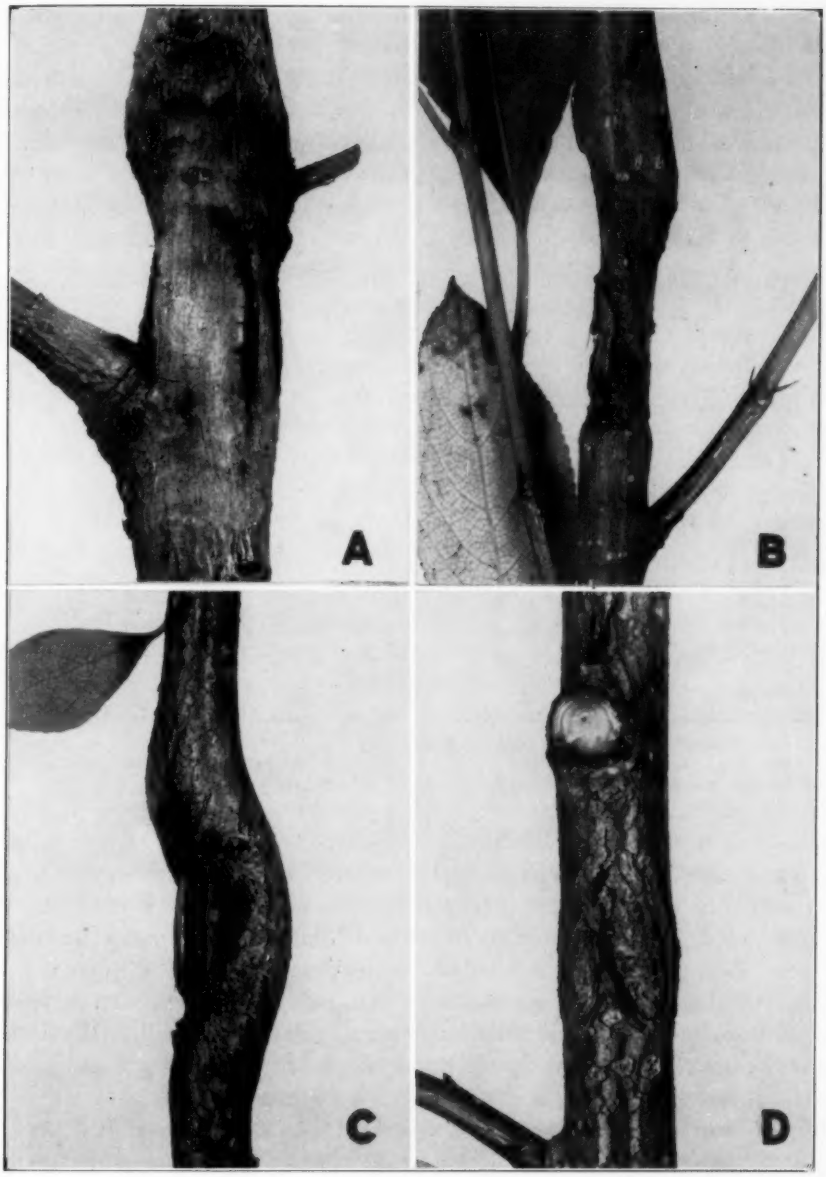

FIG. 1. Cankers caused by *Septoria musiva* on clones of hybrid poplars. A. Canker on a 3-year-old stem of a plantation tree. ×¾. B. Girdling canker from stem wound inoculation on clone OP–2. ×1. C. Canker from stem wound inoculation on clone OP–46. ×1. D. Stem wound in inoculation control on clone OP–4, showing normal healing. ×1. Photographs by R. P. Marshall.

on clone OP–55. However, variations in susceptibility were indicated by the conspicuous blackening of the bark surrounding the wounds on clones OP–2, 46, 47, and 52, in contrast to a very slight discoloration on clones OP–4, 11, 12, and 41. The wound on clone OP–55 had not healed and the stem was

slightly depressed, but no bark discoloration was evident and the tissue cultures remained sterile.

The leaf spots resulting from the inoculations corresponded with those described and illustrated by Bier (1), and mature pycnidia with spores extruding in pinkish cirri were present about three weeks after inoculation.

Table 1 gives a summary of the results from the three series of inoculations with the isolate from the Attica specimens. In all controls the wounds healed rapidly and normally (Fig. 1, D).

TABLE 1.—*Results of inoculations of hybrid poplar clones with Septoria musiva*

Clone	Part inoculated					
	Stem wound		Leaves		Petiole	
	Inoc.[a]	Inf.[a]	Inoc.	Inf.	Inoc.	Inf.
	No.	*No.*	*No.*	*No.*	*No.*	*No.*
OP–1	4	1	2	1	2	0
OP–2	4	4	2	1[b]	2	0
OP–4	4	1	2	2	2	1
OP–11	4	1	2	1	2	0
OP–12	4	1	2	0[b]	2	0
OP–41	4	1	2	0[b]	2	0
OP–46	4	3	2	1	2	0
OP–47	4	3	2	2	2	1
OP–52	4	3	2	0	2	0
OP–55	4	0	2	0	2	0

[a] Inoc. = inoculations. Inf. = infections.

[b] One leaf inoculation of clone OP–2 and OP–12, and both leaf inoculations on clone OP–41 were pruned by mistake before the results were observed.

DISCUSSION

The results from these few inoculations are not considered to be conclusive evidence of variations in susceptibility to stem infection among the clones tested, but they seem to indicate a high degree of susceptibility in clones OP–2, 46, 47, and 52. Of these, the three latter clones were derived from hybrids with *Populus maximowiczii* as one of the parents, and *P. berolinensis* as the other parent in two cases. The susceptibility of *P. maximowiczii* has not been tested, but *P. berolinensis* proved to be susceptible in the inoculations made by Bier (1). The high susceptibility of clone OP–2, with a parentage of *P. nigra* × *P. laurifolia*, indicates the desirability of testing these two parent species. However, clones with the same parentage may vary in susceptibility, as shown by the variation in inoculation results between clone OP–2 and clones OP–1 and 4. Bier also found that the Northwest poplar, a native hybrid, showed a high percentage of infection, equal to that of the exotic species *P. rasumowskyana*, while the native species from which the Northwest poplar was derived were resistant. He pointed out that *Septoria musiva* is an example of a native fungus of minor importance as the cause of a leaf spot on native species, which may act as a virulent parasite on new hosts, such as hybrids and exotic species.

The actual distribution of *Septoria musiva* in cankers on the various exotic species of poplar in the United States is not known. Apparently there have been no reports in literature of a similar canker disease other than those already mentioned from North Dakota. However, the prevalence of secondary fungi such as *Cytospora, Fusarium,* and *Phomopsis,* on cankers in the plantations and following inoculations, and the difficulty of isolating *S. musiva* from cankers thus infected, suggest the possibility that the disease may have been overlooked. In Canada (4) a perennial canker of unknown origin was described on Russian poplars, from which a species of *Cytospora* was isolated. The fungus was found particularly in callus tissue surrounding wounds and was considered to be responsible for the continuance of the disease. Moreover, Bier (1) stated that *Cytospora* was frequently isolated from the margins of older cankers that therefore might be the result of combined infection by *S. musiva* and the *Cytospora.* Pycnidia and spores of *S. musiva* are apparently produced during a very short period, even on the most susceptible trees. Infection by the fungus retards callus formation and, on the more resistant trees, creates conditions particularly favorable for infection by rapid-growing secondary fungi. This is evident from the fact that all of the wounds made in the controls of the inoculation series healed rapidly, with no infection by any fungus. It is possible, therefore, that on slightly susceptible poplars the growth of *S. musiva* might be retarded or even prevented by species of *Cytospora* or similar fungi.

Dothichiza populea, the cause of a disease of poplars, common in this country and Europe, was not found in any of the cankers examined from the New York plantations. However, clones of 10 of the hybrid poplars developed by Schreiner and Stout (11) were tested by Van Vloten (13) for susceptibility to *D. populea.* His results indicate that the clones most susceptible to that fungus were those of parentage similar to OP–2, 46, 47, and 52, which also proved highly susceptible to *Septoria musiva.*

SUMMARY

A canker disease of hybrid poplar clones in two plantings in New York State and in a planting made by the Tennessee Valley Authority at Norris, Tennessee, is caused by *Septoria musiva.* The fungus is indigenous in North America, occurring on living leaves of various species of native and exotic poplars in the United States and Canada. The canker disease has previously been reported only on exotic and hybrid poplars in Canada and Argentina.

Infection takes place through uninjured leaves and petioles, or through twig wounds. Cankers are formed on twigs of the current season's growth and pycnidia and spores appear soon after infection. The growth of the fungus from the twigs into the main stem results in the formation of cankers that, in highly susceptible trees, eventually girdle the stems. On less susceptible trees, the cankers may become infected by secondary fungi, such as *Cytospora,* which grow more rapidly than the *Septoria* and tend to mask the presence of the causal organism.

The relative susceptibility of 10 clones of hybrid poplars that have proved particularly adaptable for reforestation was tested by inoculations in the greenhouse and outdoors. Infection occurred in uninjured leaves and petioles, and in stem wounds. The results indicated a high degree of susceptibility in one clone with a parentage of *Populus nigra* × *P. laurifolia*, two of *P. maximowiczii* × *P. berolinensis*, and one of *P. maximowiczii* × *P. nigra* var. *plantierensis*.

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THE ANTIGENICITY OF SOUTHERN BEAN MOSAIC VIRUS

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(Accepted for publication September 25, 1945)

There is now abundant evidence that certain plant viruses are capable of acting as antigens and that their serological reactions are useful not only in ascertaining their relationships but also in providing a rapid means for their identification and determination of their concentration (1).

Recently, when southern bean mosaic virus (*Marmor laesiofaciens* Zaumeyer and Harter (4)) was isolated in pure form (2), it seemed desirable to test its antigenicity and to determine its serological relationship, if any, to certain other plant viruses. This paper presents the results of studies carried out on the precipitin reaction of the virus with these objectives in mind.

Unless otherwise specified the procedure in the experiments was as follows: Two-tenths cc. of the appropriate dilution of antiserum was mixed with 0.2 cc. of a chosen dilution of antigen in a 50×6 mm. test tube. All the mixtures in an experiment were examined immediately after all the tubes had been set up. They were incubated at 37° C. and were reexamined after 1 hour and again after 5 hours at this temperature. Dilutions were made in 0.85 per cent NaCl solution. The concentrations given for antigen and antiserum are those in the reacting mixture.

In a preliminary experiment mixtures were prepared containing as one component about 0.4 mg./cc. of purified southern bean mosaic virus and as the second component antiserum at 1:15 for one or another of the following viruses: Potato ringspot (*Marmor dubium* H. var. *annulus* H.), potato vein-banding (*M. cucumeris* H. var. *upsilon* H.), tobacco mosaic (*M. tabaci* H. var. *vulgare* H.), tobacco etch (*M. erodens* H. var. *vulgare* H.), tomato bushy stunt (*M. dodecahedron* H.), and tobacco necrosis (*M. lethale* H.). These antisera were the only ones available at the time. Readings were made after keeping the tubes at room temperature for 1 hour and 4 hours and then after keeping them overnight at 7° C. The southern bean mosaic virus reacted with none of the antisera although each antiserum reacted strongly in control mixtures containing its homologous virus in plant juice cleared by low speed centrifugation. The experiment indicated that southern bean mosaic virus was not serologically related to any of these viruses.

Because southern bean mosaic virus resembled bushy stunt virus and tobacco necrosis virus in some of its properties a purified preparation of southern bean mosaic virus was then tested at concentrations of 0.5, 0.25, 0.125, 0.063, 0.031, 0.016, 0.008, 0.004, 0.002, and 0.001 mg./cc. against 1:18 dilutions of tomato bushy stunt and tobacco necrosis antisera and a 1:18 dilution of normal rabbit serum. There was no precipitin reaction at any dilution of the virus, again suggesting that southern bean mosaic virus was serologically distinct from tomato bushy stunt and tobacco necrosis viruses.

The next step was to prepare an antiserum to southern bean mosaic virus. The virus preparation used as antigen had been partially purified and concentrated by precipitating it with $(NH_4)_2SO_4$, removing the precipitate with low-speed centrifugation, and redissolving it in water. It was then further concentrated and purified by centrifuging it twice at 24,000 r.p.m. for 2½ hours to sediment the virus, taking it up in water after each centrifugation and centrifuging at 3000 r.p.m. for a few minutes to remove aggregated colloidal material. It was finally dialyzed against distilled water. The concentrated material contained 84 mg./cc. of virus and had an amber color at this concentration. It was found to be essentially homogeneous when tested in an analytical centrifuge, a Tiselius electrophoresis apparatus, and a diffusion apparatus.[1] Examination under an electron microscope showed it to consist of spherical particles of uniform size and to be essentially free of extraneous particles of other shapes or sizes (3).

TABLE 1.—*Titration of antisera to southern bean mosaic virus*

Serum	Serum dilution	Time (minutes)	Antigen concentration (mg./cc. of virus)	
			0.1	0.01
Antiserum from rabbit No. 1	1: 10	15	+	−
		135	++++	±
		1440	++++	+
	1: 100	15	+	−
		135	++	−
		1440	+++	++
Antiserum from rabbit No. 2	1: 10	15	+	−
		135	++++	±
		1440	++++	++
	1: 100	15	+	−
		135	++	−
		1440	+++	++

As a preliminary, 5 cc. of blood were withdrawn from the ear vein of each of the two rabbits to be used later in preparing the antisera. This blood furnished the normal sera which were to be used later in comparison with the immune sera. After waiting a week, each of the two rabbits was injected intraperitoneally with 2 mg. of virus dissolved in 4 cc. of 0.85 NaCl solution. After a ten-day interval each of the rabbits was bled, and the serum thus obtained was held at – 10° C. until needed.

A purified preparation of southern bean mosaic virus was tested against the normal serum and the antiserum from each of the two rabbits. The mixtures were kept at 37° C. for 5 hours, after which they were kept at 7° C. for the next 19 hours. The reactions with the antisera are shown in table 1. Since there was no reaction of virus with either normal serum when tested at the same dilutions used for the antisera, nor any reaction when antisera were diluted 1: 1000 or antigen diluted to 0.001 or 0.0001

[1] Data to be published.

mg./cc. of virus, these negative results are not included in the table. The data show that southern bean mosaic virus is antigenic and suggest that the serological reaction can be useful in testing for its relationships.

The next step was to test the specificity of the southern bean mosaic virus antiserum. The two viruses that most closely resemble southern bean mosaic virus in properties, tobacco necrosis and tomato bushy stunt viruses, were used in the tests. Purified preparations of these viruses were prepared by differential centrifugation and the final products were analyzed for protein content. A series of dilutions of each of these purified preparations and of a purified preparation of southern bean mosaic virus were tested against normal serum and against antiserum to each of the three. The dilution of antiserum in each mixture was 1:20. The data obtained are in table 2.

After adding the solutions of antigen and antiserum to all of the tubes and before mixing them, they were examined. Some tubes showed a definite cloudy disc where antiserum and antigen came in contact. Such a reaction is indicated in the table by a "+" sign in the space allotted to the 15-minute reading. The final data show that southern bean mosaic virus reacted with homologous antiserum, prepared by means of a single intraperitoneal injection of 2 mg. of virus, at a concentration as low as 0.01 mg./cc. but failed to react with the heterologous antisera at any concentration tested. Tobacco necrosis virus reacted only with its homologous antiserum and at a concentration as low as 0.05 mg./cc. Similarly, tomato bushy stunt virus reacted only with the homologous antiserum and at a concentration as low as 0.02 mg./cc. A second test set up in essentially the same manner gave practically identical results. These results indicate that each of the viruses is serologically distinct.

The data thus far presented show definitely that southern bean mosaic virus is antigenic and that its precipitin reaction is specific, so far as tested. These data were obtained entirely from tests made with purified preparations of virus. It seemed desirable to determine whether the juice from diseased bean plants would give a specific serological reaction when mixed with antiserum to southern bean mosaic virus. Juices expressed from ground, unfrozen, healthy and diseased Bountiful bean (*Phaseolus vulgaris* L.) plants were centrifuged for a few minutes at 3000 r.p.m. The juices were then tested at a series of dilutions in mixtures containing normal serum or antiserum to southern bean mosaic virus at a dilution of 1:20. After 1 hour at 37° C. there were specific precipitin reactions in the tubes containing homologous antiserum and diseased juice at dilutions 1:2, 1:4, 1:8, 1:16, and 1:32. No reaction occurred in control tubes containing juice from healthy plants and the antiserum or in those containing diseased juice and normal serum. After 5 hours at 37° C. the diseased juice up to a dilution of 1:128 exhibited a reaction with antiserum but by this time artifact reactions were developing in all tubes containing juice, whether diseased or healthy, at dilutions up to 1:8. A second test gave essentially the same results. These experiments show clearly that the antiserum reacts specifi-

TABLE 2.—*Specificity of antiserum to southern bean mosaic virus*

Serum[a]	Time (minutes)	Antigen concentration (mg./cc. of virus)				
		0.1	0.05	0.025	0.0125	0.0063[b]
Antigen = southern bean mosaic virus						
Normal	15	−	−	−	−	−
	60	−	−	−	−	−
	300	−	−	−	−	−
Anti-southern bean mosaic virus	15	+	+	+	+	−
	60	++	+	+	+	−
	300	++++	+++	++	+	±
Anti-tobacco necrosis virus	15	−	−	−	−	−
	60	−	−	−	−	−
	300	−	−	−	−	−
Anti-tomato bushy stunt virus	15	−	−	−	−	−
	60	−	−	−	−	−
	300	−	−	−	−	−
Antigen = tobacco necrosis virus						
Normal	15	−	−	−	−	−
	60	−	−	−	−	−
	300	−	−	−	−	−
Anti-southern bean mosaic virus	15	−	−	−	−	−
	60	−	−	−	−	−
	300	−	−	−	−	−
Anti-tobacco necrosis virus	15	+	+	−	−	−
	60	+	+	−	−	−
	300	++	+	±	−	−
Anti-tomato bushy stunt virus	15	−	−	−	−	−
	60	−	−	−	−	−
	300	−	−	−	−	−
Antigen = tomato bushy stunt virus						
Normal	15	−	−	−	−	−
	60	−	−	−	−	−
	300	−	−	−	−	−
Anti-southern bean mosaic virus	15	−	−	−	−	−
	60	−	−	−	−	−
	300	−	−	−	−	−
Anti-tobacco necrosis virus	15	−	−	−	−	−
	60	−	−	−	−	−
	300	−	−	−	−	−
Anti-tomato bushy stunt virus	15	+	+	+	−	−
	60	++	+	+	−	−
	300	+++	++	+	±	−

[a] The serum in each mixture was diluted 1 : 20.

[b] Negative results were obtained with virus concentrations of 0.0031 and 0.0016 mg./cc. and accordingly mixtures with these concentrations were omitted from the table.

cally with the juice from diseased plants but not with that from healthy plants. The experiments also indicate that the original purified virus used in preparing the antiserum was essentially free of protein constituents of the normal plants.

The results here reported are of interest not only because they show that southern bean mosaic virus is antigenic and that the precipitin reaction can be of use in testing for its virus relationships but also because they bring additional evidence for the belief that southern bean mosaic virus is an independent and distinct virus species. It has been shown elsewhere (3) that this virus can be distinguished from most other plant viruses by its high degree of thermostability and that in plant protection tests it is immunologically distinct from tobacco mosaic and tobacco necrosis viruses, both of which it resembles in thermostability. Hitherto, it was differentiated from tomato bushy stunt virus mainly on the striking differences in host range but partly also because of differences in physical and chemical properties. Southern bean mosaic virus has now been demonstrated to be distinguishable serologically from tomato bushy stunt virus.

SUMMARY

It is shown that southern bean mosaic virus is antigenic and that its precipitin reaction is useful in distinguishing it from other viruses. Southern bean mosaic virus is serologically distinct from tobacco necrosis virus and tomato bushy stunt virus, which it resembles in some of its physical properties. It is also serologically distinct from potato ringspot, potato veinbanding, tobacco mosaic, and tobacco etch viruses. Its antiserum, prepared with purified virus, reacts specifically with juice from Bountiful bean plants infected with the virus. The data bring additional evidence for the view that southern bean mosaic virus is an independent and distinct virus species.

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PHYTOPATHOLOGICAL NOTES

Arasan for Control of Fungi in Germinating Corn Seed.[1]—That Arasan inhibits the development of *Diplodia zeae* (Schw.) Lév. has been shown by Hoppe[2] in a study of infected corn seeds placed on agar surfaces. He found that Arasan was equal to Barbak C and Semesan Jr. and superior to Spergon in retarding the growth of *D. zeae*. There is also evidence[3] that in laboratory germination tests *D. zeae* is more effectively controlled with Arasan than with dusts containing ethyl mercury phosphate, ethyl mercury p-toluene sulfonanalide, or 2–3 dichloro, 1–4 naphthoquinone. Arasan applied to samples of low-germinating corn at the rate of 0.15 per cent by weight, however, did not control *Rhizopus nigricans* Ehr. as successfully as it did *D. zeae*. In contrast to these results, heavier dosages of Arasan prevented *R. nigricans* and other molds from growing on germinating seeds of cucumber, muskmelon, and squash.[4]

Apparently a proper dosage of Arasan should control both pathogenic and saprophytic fungi on germinating corn seed. To obtain information on this subject six selected dealers' lots of corn seed infested with *Diplodia zeae*, *Fusaria*, and *Rhizopus nigricans* and four lots infested with *R. nigricans* were treated with certain mixtures of Arasan and flour.

The mixtures (Table 1) contained 100, 67, 50, and 33 per cent Arasan and 0, 33, 50, and 67 per cent, respectively, of wheat flour. A known weight of seeds was agitated with one of the dusts for three minutes. The material that did not adhere was removed by shaking the treated seeds in a sieve. Arasan adhered at the rates of 2.3, 1.3, 0.9, and 0.6 ounces per bushel of seed for the 100, 67, 50, and 33 per cent Arasan mixtures, respectively.

The treated seeds and the controls were counted into replicates of 100 and were either germinated at once or stored in a dry laboratory for one, two, or three months before being germinated. All seeds were germinated on moistened paper towels at alternating temperatures of 20° and 30° C. Records were taken during the sixth day of germination.

Neither Arasan alone nor any of the Arasan-flour mixtures retarded the germination of any seed lot. Approximately 90 per cent of the live seeds had formed normal seedlings after only three days on the moistened towels regardless of the dosage of Arasan or the length of the storage period. In germination the control excelled any one treatment in only 29 of the 140 separate tests. The average results from all of the tests showed that the percentage germination of untreated seed was not higher than that of seed

[1] Journal Paper No. 613 of the New York State Agricultural Experiment Station, Geneva, New York.

[2] Hoppe, P. E. Comparison of certain mercury and non-metallic dusts for corn seed treatment. Phytopath. **33**: 602–606. 1943.

[3] Crosier, Willard. Chemical control of molds, bacteria and fungus pathogenes on cucurbit, pea and sweet corn seeds. Proc. Assn. Off. Seed Analysts **1943**: 38–41. 1945.

[4] Crosier, Willard. Chemical seed protectants used as disinfestants during germination of cucurbit seeds. News Letter Assn. Off. Seed Analysts **18**(2): 3–6. 1944.

treated with any Arasan mixture or with Arasan alone. This was true for all ten lots of corn.

Rhizopus nigricans is readily controlled in viable, fairly clean seed (Table 1). In heavily infested seed containing 8 to 20 per cent dead kernels, however, *R. nigricans* appeared to be beneath the pericarp of some seed and hence not exposed to surface sterilization. The heavier concentrations of Arasan (100 and 67 per cent) excelled the lighter concentrations (50 and 33 per cent) in controlling fungi in this type of corn seed.

While neither *Diplodia zeae* nor *Fusaria* were present in a high percentage of seeds, it is apparent (Table 1) that Arasan retarded the develop-

TABLE 1.—*Laboratory germination, seedling weight, and fungus contaminants of corn seed treated with certain mixtures of Arasan and wheat flour*

Arasan-flour mixture	Normal seedlings		Seeds or seedlings contaminated with						Green weight of seedlings in entire test	
			Rhizopus nigricans		*Diplodia zeae*		*Fusaria*			
	Pct.	*Rank*[a]	*Pct.*	*Rank*	*Pct.*	*Rank*	*Pct.*	*Rank*	*Grams*	*Rank*
Averages for six seed lots infested with Diplodia, Fusaria, and Rhizopus[b]										
100–0	90	66	4	75	Tr.	81	1	59	78	54
67–33	90	57	5	76	Tr.	67	1	61	79	64
50–50	90	64	11	62	Tr.	59	1	67	80	66
33–67	88	46	27	32	1	35	1	53	79	56
0–100	86	17	48	5	5	8	4	10	72	10
Averages for four seed lots infested with Rhizopus[c]										
100–0	97	27	Tr.	26	.....	.....	.....	.....	78	21
67–33	96	15	0	29	.....	.....	.....	.....	78	23
50–50	97	24	Tr.	27	.....	.....	.....	.....	78	24
33–67	97	22	1	18	.....	.....	.....	.....	79	27
0–100	96	12	21	0	.....	.....	.....	.....	72	5

[a] Rank is based upon total number of items in which an Arasan-flour mixture (or control) proved superior to any other mixture (or control). Normally each mixture (and control) would be ranked from 0 to 4 in ascending order of superiority.

[b] Twenty-six replicated tests; 100 possibilities for superiority.

[c] Twelve replicated tests; 40 possibilities for superiority.

ment of these fungi. When these fungi did develop on Arasan-treated seed the colonies were markedly smaller than those developing from untreated seed.

Chemical injury either was not apparent or was very slight. In a bulk lot of surface-cracked seeds treated with Arasan alone 2 per cent of seedlings were slightly injured. No injury was noticed in germinating seeds of other corn lots.

The average green weights of seedlings in the 100-seed tests prove that Arasan either did not cause any chemical injury or the increases in weight attributable to control of fungi were greater than the decreases effected by chemical injury. The data in table 1 show, furthermore, that the treatments not only did not depress, but actually resulted in increased green weights of the seedlings. The data do not indicate, however, whether the increases in green weight are due to control of the fungi, the effect of the toxicant

content of Arasan, or the effect of the diluent present in Arasan and the Arasan-flour mixtures upon the seedlings.

In two other bulk lots of seeds carrying only traces of fungi the average green weights of seedlings from ten replicated tests of each treatment were: 100 per cent Arasan—76 grams, 50 per cent Arasan—76 grams, 33 per cent Arasan—77 grams, and no treatment—72 grams. It does not appear probable that the differences in weights between the treated and untreated seeds were caused by control of fungi alone.—WILLARD CROSIER, Assistant Professor, Division of Seed Investigations, Cornell University, Geneva, New York, and STEWART PATRICK, Research Associate, Division of Seed Investigations, Cornell University, Geneva, New York.

Internal Brown Spot, a Boron Deficiency Disease of Sweet Potato.[1]—In exploring certain possible causes of internal cork,[2] a newly described disease of sweet potato (*Ipomea batatas* (L.) Lam.), the likelihood that boron deficiency, or some other nutritional agency, might be involved was investigated in 1944. In one field experiment of complex design sweet potatoes of the Porto Rico variety were grown on replicated field plots, located on both limed and unlimed soils at three different levels of potassium fertilization and supplied with borax at rates of 0, 5, 10, 20 and 30 pounds per acre. Although there was no correlation between the incidence of internal cork and the response of the sweet-potato plants to these nutritional treatments, this experiment, nevertheless, afforded an opportunity for studying certain physiogenic abnormalities of plants grown on no-borax plots. The induced disease is herein referred to as "internal brown spot" because it is characterized primarily by the occurrence of brown necrotic areas in the flesh of the roots. Inasmuch as the trouble has not been observed on plants supplied with borax, it has been attributed to boron deficiency. This report deals only with field symptoms of the disease and is offered preliminary to a more comprehensive paper to be published upon the completion of work now in progress. A brief description of some of the symptoms noted herewith has already been published.[3]

The symptoms on the vines and leaves were not detected until the latter part of August, about two months after planting, when some of the plants on no-borax plots showed a restriction of terminal growth and shortening of the internodes. As the season advanced these symptoms developed in varying degree on nearly all of the plants on all no-borax plots. As the disease continued to develop, the petioles became curled and the terminals became stunted and distorted. In October many of the terminals died and vine growth ceased. The older leaves turned yellow and shed from the vines, exposing the crowns of the plants. Partial collapse of vines near the crown,

[1] Technical contribution number 128 of the South Carolina Agricultural Experiment Station.

[2] Nusbaum, C. J. Internal cork, a new disease of sweet potato of unidentified cause. Phytopath. 36: 18–23. 1946.

[3] Nusbaum, C. J. Studies of the use of boron with sweetpotatoes. S. C. 57th Ann. Rept., 144–149. 1945.

probably due to sunburn, was not uncommon. A comparison of vines on boron-deficient and healthy plants is shown in figure 1. These photographs were taken in mid-October just before harvest. Both plants represented

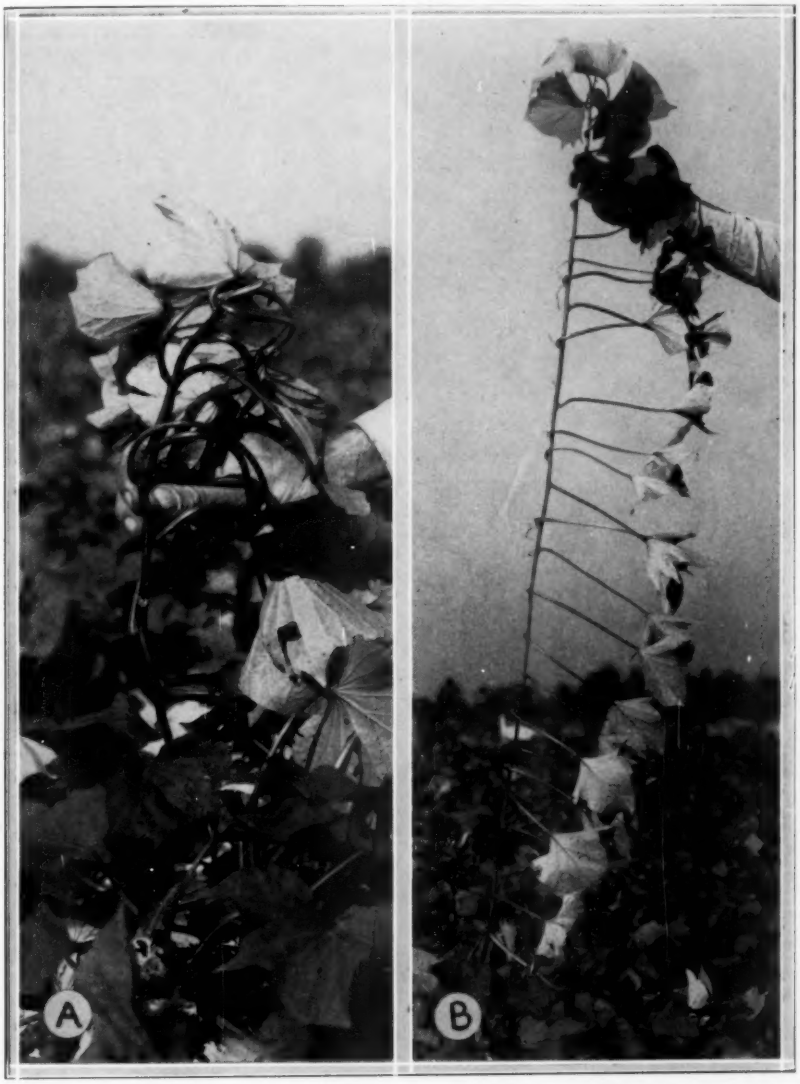

FIG. 1. Comparison of vine growth on boron-deficient and healthy plants, both of which were grown in a high-calcium, high-potassium block. A. Plant grown upon no-borax plot. Note the gnarled growth characterized by shortened internodes, curled petioles, defoliation of basal portion of the vine, and distorted terminal. B. Plant grown upon a plot supplied with borax at the rate of 10 pounds per acre. Note the long unrestricted vine growth, the more or less regularly spaced nodes, and the straight petioles.

were in the high-lime, high-potassium block. The deficient plant (A) shows the typical gnarled growth characterized by short, irregular internodes, curled petioles, dead terminal and abscissed lower leaves. The healthy plant

(B), which received borax at the rate of 10 pounds per acre, shows long, unrestricted vine growth with more or less regularly spaced nodes and straight petioles. At harvest the terminals of such vines were still elongating.

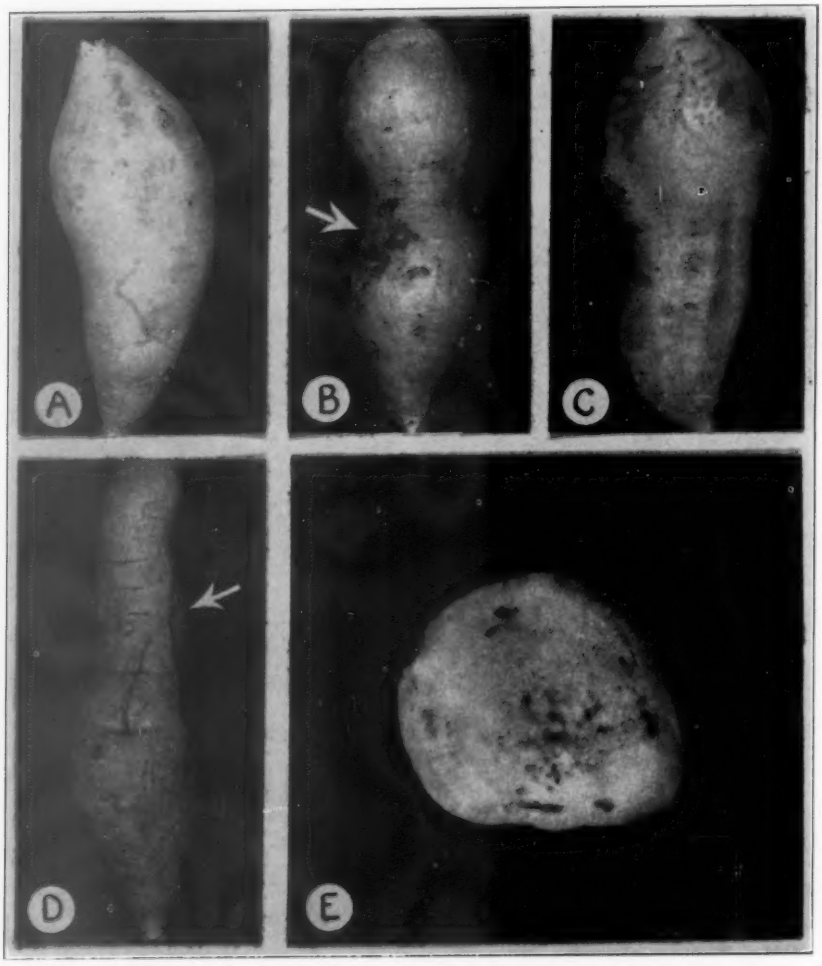

FIG. 2. Sweet-potato roots produced in a low-calcium, high-potassium block. The healthy root (A) was produced on a plot which received borax at the rate of 5 pounds per acre. Note the symmetrical shape and smooth appearance. The external appearance of typical diseased roots is shown in a dumbell-shaped root (B) with an arrow pointing to an exudate-covered canker, a lop-sided root (C), and a spindling root (D) with a long surface canker, without exudate, indicated by an arrow. E. Cross section of a diseased root from a no-borax plot. Note the uneven outline of the root and the appearance of the indistinct spotting, especially in the cambial zone near the periphery.

At digging time the roots from no-borax plots showed varying degrees of both external and internal degeneration. In outward appearance they were misshapen and the skin was rough and leathery in texture somewhat resembling buckskin. Many of the roots appeared not to have filled out at certain

points, resulting in dumbell-shape, lopsided or spindle-shape malformations, such as those shown in figure 2, B, C and D. A smooth, well-formed root taken from a plot which received 5 pounds of borax per acre is shown in figure 2, A, for comparison. Severely affected roots usually showed surface cankers which were sometimes covered with a hardened and blackened exudate (Fig. 2, B), the origin or nature of which has not been ascertained. The fleshy root shown in figure 2, D, however, shows an elongated canker, indicated by an arrow, without this exudate. Roots with the external symptoms described usually, but not always, had a characteristic internal breakdown, as shown in figure 2, E. It always was found, however, in severely misshapen roots or in those with surface cankers.

The internal necrotic areas were variable in size and brown with indistinct margins. They occurred indiscriminately throughout the flesh but seemed to be most prevalent in the cambial zone near the periphery of the root. The origin of the spots or the tissues involved has not been determined. The flesh of roots with internal brown spot was generally of poorer color and of softer texture than that of healthy roots.

The possible nutritional rôle of boron in the sweet potato was first reported by Willis,[4] who pointed out that small applications of borax often prevented cracking of the fleshy roots and improved the flavor and texture of the flesh. He also suggested that darkening of the sweet potatoes, hitherto attributed to damage from chilling at temperatures above freezing, could be eliminated or reduced by the use of borax in the fertilizer. In the studies here reported cracked roots occurred in small amounts in nearly all plots regardless of rate of borax applied. Furthermore, the internal brown spot symptom here described developed in sweet potatoes which had not been exposed to temperatures below 50° F., either in the field or in storage, and was distinctly different from the discoloration and subsequent breakdown of the flesh commonly attributed to chilling injury. Reave *et al.*[5] state briefly that sweet-potato plants grown in the greenhouse without boron produced roots having "dark streaked centers" but clear-cut boron deficiency symptoms were not observed in the field.—C. J. NUSBAUM, Edisto Experiment Station, Branch of Clemson College, Blackville, S. C.

A Rapid Method for Isolating Single Ascospores from Apothecia.—This note deals with a subject with which the writer has not been in contact for many years but it is impelled by the recent death of Professor H. H. Whetzel. While serving as his assistant in 1917 the writer discovered a rapid method for isolating single spores from the apothecia of various species of *Sclerotinia* and related genera. The procedure for getting single-spore cultures as taught the writer by Professor Whetzel consisted in suspending an apothecium within and from the top of a large bell-jar so that the hymenial disc

[4] Willis, L. G. Apply borax to improve quality of sweet potatoes. N. C. Agr. Exp. Sta. Special Circ. 1. 1943.

[5] Reave, Eldrow, A. L. Prince, and F. E. Bear. The boron needs of New Jersey soils. N. J. Agr. Exp. Sta. Bul. 709. 1944.

faced downward. An open Petri dish containing nutrient agar was then placed within and at the base of the bell-jar to catch the spores that floated down. The apparatus was left thus overnight. Later, the Petri dish was examined microscopically and young cultures from individual spores were transferred to tubes. This method got results but it was laborious and favorable to contaminations. Not infrequently only very few ascospores were shed. In an effort to improve the method, the writer tried immersing the lower two-thirds of an apothecium in 95 per cent alcohol. Immediately a cloud of ascospores was ejected. Taking advantage of this action, single ascospores were isolated simply by placing three Petri dishes containing nutrient agar near a vial with enough 95 per cent alcohol to cover the lower two-thirds of the apothecium when this was dropped into the vial. In order not to interfere with spore dispersal, vials were selected that were not much taller than the fruiting bodies and in order to keep the apothecia in an upright position to avoid getting alcohol on the hymenium, vials were selected that were only slightly larger in diameter than the apothecia. Immediately following immersion and the ejection of a cloud of ascospores, a Petri dish was momentarily opened in the cloud. This was quickly repeated with the second and third Petri dishes. Usually the last caught only a few scattered spores in perfect placement for isolation. Due to their short exposures, the plates usually were wholly free from contaminations.

Among the many apothecia collected by Professor Whetzel were small ones, only a few millimeters in diameter, from which it was very difficult to obtain spores by the bell-jar method. In applying the principles of the new technique, forceps were used to hold these apothecia face downward over nutrient agar in an open Petri dish. A needle that had been dipped in alcohol then was lightly rubbed against the outer layer of the fruiting body. This brought about an immediate ejection of ascospores.

The advantages of the new method are rapidity, excellent spore dispersal, freedom from contamination, and applicability to the smallest apothecia. Possibly the method may be applicable to some other fungus fruiting bodies.—V. F. TAPKE, Plant Industry Station, Beltsville, Maryland.

Induced Baldhead in Soybean.[1]—For five years the Iowa State College Seed Laboratory has been investigating the possibility of indexing commercial lots of seed for their reaction to low temperature in Pythium-infested soil at different moisture levels in terms of their response when planted in the field under conditions unfavorable to germination. In addition, a study has been made of the relative ability of seed protectants to prevent seed decay and seedling blight when seeds are germinated in Pythium-infested soil. In April, 1944, treated and nontreated seeds of Bansei soybean were planted in a mixture of Pythium-infested soil and sand with moisture content of about 15 per cent, according to the procedure described by Rice[2] and

[1] Journal Paper No. J-1318 of the Iowa Agricultural Experiment Station, Ames, Iowa. Project No. 427.

[2] Rice, N. W. The development and evaluation of seed laboratory methods for determining the presence and significance of certain soil-borne and seed-borne organisms. Unpublished doctor's thesis, Iowa State College Library. 1944.

TABLE 1.—*Percentages of normal and "baldhead" seedlings from treated and nontreated soybean seed planted in Pythium-infested soil, Iowa, 1945*

Treatment	7 days cold soil				10 days cold soil			
	Kanro		Lincoln		Kanro		Lincoln	
	N[a]	B[a]	N	B	N	B	N	B
Nontreated	49	8.2	43.8	39.2	52.8	21.8	39.5	37.2
Sem. Jr.	70	7.2	72.8	19.5	.....	.....	.....	.....
Spergon	83	1.2	95.0	2.2	.....	.....	.....	.....
Arasan	85	0.2	98.2	0.5	89.8	0.5	95.8	0.75

[a] N = Normal seedlings, B = Baldheads.

Porter.[3] The soil used was naturally infested with *Pythium graminicola* Subr. and *P. de Baryanum* Hesse. The flats containing the seeds were placed in a room with a constant temperature of 10° C. for 7 days, then transferred to an adjoining room at 26° to 28° C. At the end of 5 days in the latter room the seedlings were examined and classified as emerged, normal, and abnormal. Fungicides used were Arasan, Spergon, and Fermate.

Among the seedlings classed as abnormal were a number, produced by nontreated seed, that resembled "baldheads."[4, 5] Close examination revealed

FIG. 1. Seedlings of Lincoln soybean grown in Pythium-infested soil at 10° C. for seven days followed by exposure at 26–28° C. for five days. Left, treated with Arasan. Right, not treated.

[3] Porter, R. H. Testing the quality of seeds for farm and garden. Iowa Agr. Exp. Sta. Res. Bul. 334. 1944.

[4] Borthwick, H. A. Thresher injury in baby Lima beans. Jour. Agr. Res. [U.S.] **44**: 503–510. 1932.

[5] Harter, L. L. Thresher injury a cause of baldhead in beans. Jour. Agr. Res. [U.S.] **40**: 371–384. 1930.

that the plumule had failed to develop because necrosis of the primary leaves had occurred early in the growth of the plumule. In many cases axillary buds had developed at the base of the decayed plumule in seedlings that were three or four inches high. Of 400 nontreated seeds planted, 18.2 per cent produced "baldheads." The percentage of baldheads from treated seed was 1.4 for Arasan, 0.65 for Spergon, and 4.4 for Fermate. This particular lot of seed when tested in sand at 26° to 28° C. gave 99 per cent normal seedlings with no baldheads.

In April, May, and June, 1945, seeds of Kanro and Lincoln soybeans from the 1944 crop were selected for test. Tests in sand at 26°–28° C. gave 95.5 per cent normal seedlings for Kanro and 98.0 per cent normal for Lincoln. No "baldheads" were produced. Treated and nontreated seed of these two lots was then planted in Pythium-infested soil and held for 7 or 10 days at 10° C., then transferred to a room at 26°–28° C. The counts of emerged seedlings gave results as shown in table 1.

In figure 1 are shown a few normal and "baldhead" seedlings of the variety Lincoln.

To determine the effect of low temperature alone seed of both varieties was planted in sterilized sand and the flats were held 10 days at 10° C. before transfer to the warm room. The results showed one per cent "baldhead" seedlings each from nontreated seed and Arasan-treated seed, respectively. The percentages of normal seedlings were 93 and 94 for treated and nontreated, respectively.

The data herein summarized indicate that soybean seed of high germinability when planted in Pythium-infested soil with a moisture content of 15 per cent and retained at 10° C. for 7 or 10 days may be expected to produce a high percentage of "baldhead" seedlings in which the plumule is either killed or partially decayed.—R. H. PORTER, Iowa State College, Ames, Iowa.

Relative Resistance and Susceptibility of U.S. 243 and U.S. 343 Lima Beans to Lima-Bean Mosaic.—During the summer of 1945 two promising strains of Lima beans were being increased for distribution under the designations U.S. 243 and U.S. 343. Trial plantings were also made at numerous experiment stations[1] and in a few home gardens. In June a diseased specimen of U.S. 343 apparently affected with mosaic was brought to the attention of the writers. An examination of the garden from which this plant came showed that two 20-foot rows, one of U.S. 243 and one of U.S. 343, had been planted end to end. Of the 49 plants in the row of U.S. 343, 33 had mosaic; while all of the 33 plants in the row of U.S. 243 appeared to be free of the disease. Inoculations to appropriate hosts indicated that the mosaic was caused by a virus similar to the one described by Harter.[2] Greenhouse inoculations (Table 1) substantiated the field observations. Inoculations

[1] Magruder, Roy, and R. E. Wester. A preview of two new bush lima beans. Market Growers Jour. **74**: 23. 1945.

[2] Harter, L. L. Mosaic of lima beans (*Phaseolus lunatus macrocarpus*). Jour. Agr. Res. [U.S.] **56**: 895–906. 1938.

TABLE 1.—*The relative susceptibility of U.S. 243 and U.S. 343 to lima bean mosaic*

Strain	Noninoculated		Inoculated	
	Total No. plants	No. diseased	Total No. plants	No. diseased
U.S. 243	33	0	87	0
U.S. 343	97	0	145	93

from U.S. 243 to tobacco showed that this Lima strain was resistant and not a symptomless carrier of the virus.

FIG. 1. U.S. 343, susceptible to Lima-bean mosaic.

The resistance of U.S. 243 and susceptibility of U.S. 343 (Fig. 1) is an interesting case since both these lines are selections from the same Fordhook × Sieva cross. Harter demonstrated that Fordhook is resistant to the virus; while Sieva is susceptible. Apparently in developing the two Lima strains, a resistant and a susceptible line were isolated by chance. This situation is another example[3, 4] of how, in selecting for desirable horticultural characters, genes for resistance may be lost unless particular attention is given to the pathological phases of the breeding program.

Mosaic in Lima beans has never been serious in the past, even in the case of susceptible varieties, and is therefore likely to be of only technical interest for some time.—DEAN E. PRYOR and ROBERT E. WESTER, Plant Industry Station, Beltsville, Md.

[3] Pryor, Dean E. A unique case of powdery mildew on lettuce in the field. U. S. Dept. Agr., Plant Disease Reporter **25**: 74. 1941.

[4] Whitaker, Thomas W., and Dean E. Pryor. Genes for resistance to powdery mildew in *Cucumis melo*. Proc. Amer. Soc. Hort. Sci. **41**: 270–272. 1942.